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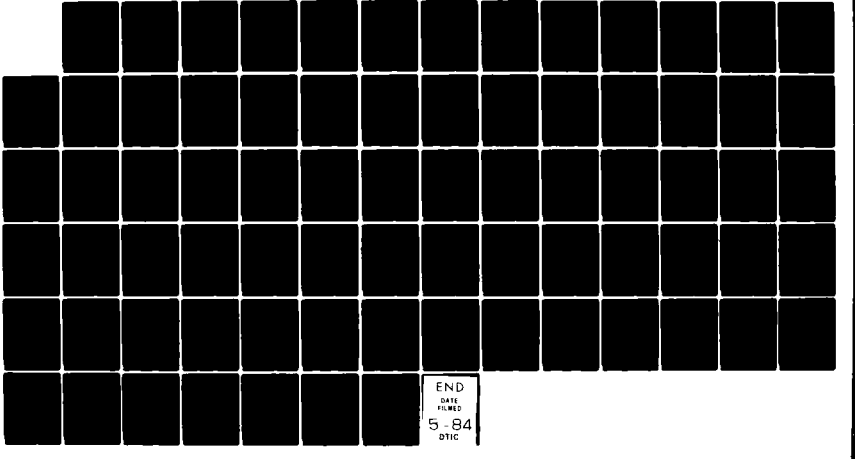

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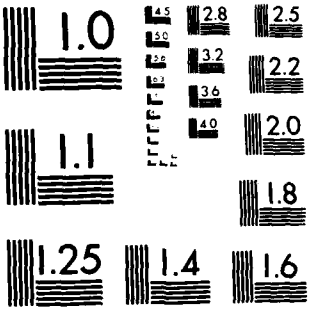

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THE SYNTHESIS AND STUDY OF NEW RIBAVIRIN DERIVATIVES AND RELATED NUCLEOSIDE AZOLE CARBOXAMIDES AS AGENTS ACTIVE AGAINST RNA VIRUSES

Annual Progress Report No. 2

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and

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September 1981

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A number of triazole, imidazole, pyrazole and purine nucleoside analogs containing a carboxamide function, related to ribavirin have been prepared and tested in vitro against several RNA viruses having substantial implications from a global epidemiological standpoint. Considerable differences in antiviral efficacy was noted depending on the type of viral infection. CONTINUED

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

In an effort to improve the antiviral potency of ribavirin, several selected derivatives of ribavirin have been prepared. A number of heterocycles and nucleosides in the 1,2,3-triazole, 1,2,4-triazole, imidazole, pyrazole and purine ring system have also been prepared to study the structure-activity relationship. New and improved synthetic methods, and isolation techniques have been developed during the course of this synthetic study. Most of the compounds synthesized were tested at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, against RVFV, VEE, PICH, YF — CONTINUED

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19. Key Words - continued.

Improved in vivo activity was observed with ribavirin 3',5'-cyclic phosphate against VEE over that of ribavirin. Several 7-deazapurine nucleosides, particularly 6-chloro-7-deazapurine riboside, exhibited very significant antiviral activity in vitro against RVFV, PICH, YF and SF, and the activity was comparable to that of pyrazofurin or tunicamycin.

20. Abstract - continued.

and SF viruses in vitro as well as in vivo. Of the compounds tested, in parallel with ribavirin, ribavirin 3',5'-cyclic phosphate exhibited superior in vivo activity against VEE. On day 20 at 25 mg/kg/day of ribavirin 3',5'-cyclic phosphate, there were 20 out of 20 survivors in the VEE-infected mice compared to 8 out of 20 survivors in the control. 9-(β-D-Ribofuranosyl)purine-6-carboxamide and several 7-deazapurine nucleosides showed very significant antiviral activity in vitro against the subject viruses. 6-Chloro-7-deazapurine riboside was shown to be superior to ribavirin against RVFV, PICH, YF and SF in vitro and the activity was comparable to that of natural nucleoside antibiotic pyrazofurin or tunicamycin. However, like pyrazofurin and tunicamycin, 6-chloro-7-deazapurin riboside was found to be toxic in vivo.

SUMMARY

Ribavirin is a broad spectrum virustatic agent and is important as a clinically useful antiviral drug. In addition to several DNA viruses, ribavirin is very effective against RNA viruses like Rift Valley fever, Lassa or Machupo, Bolivian hemorrhagic fever, etc., which have substantial implications from a global epidemiologic standpoint. The potent activity of ribavirin against Lassa fever in subhuman primate models has clearly indicated the potential human use of antiviral agents against virulent tropical RNA viral diseases. It is now quite clear from the in vitro and in vivo evaluation of ribavirin derivatives and certain azole carboxamide nucleosides prepared during the subject contract, that substantial differences in antiviral activity exist depending on the type of viral infection and the nucleoside derivative. It has been possible in certain instances to improve on the in vivo antiviral activity of ribavirin, as noted in the case of ribavirin 3',5'-cyclic phosphate against VEE.

During the past year, in a search for new antiviral agents having better potency than ribavirin with little or no host toxicity, several triazole, imidazole, pyrazole and purine nucleosides have been prepared and tested in vitro against several RNA viruses. 9-(β-D-Ribofuranosyl)purine-6-carboxamide inhibited the RVF virus growth to the extent of 90% at 250 μg/ml and inhibition was nearly complete at 500 μg/ml. It also inhibited PICH to the extent of 90% at 250 μg/ml. 9-(β-D-Ribofuranosyl)purine-6-carboxamide employed in the treatment of RVF virus infected mice at 50 mg/kg/day gave a 55% survival rate on day 21 compared to a 30% survival in the controls. Several 7-deazapurine nucleosides like 7-deazainosine, 6-chloro-7-cyano-7-deazapurine riboside and 6-chloro-7-deazapurine riboside showed significant antiviral activity against RVFV, PICH, YF and SF in vitro. 6-Chloro-7-deazapurine riboside was found to be superior to ribavirin against the subject viruses in vitro and the activity was comparable to that of pyrazofurin and tunicamycin. However, like pyrazofurin and tunicamycin, 6-chloro-7-deazapurine riboside was found to be toxic in vivo. The possibility of preparing azole nucleosides with more potency as noted with pyrazofurin is a major goal as yet unachieved. It is hoped that azole nucleosides with the antiviral potency of pyrazofurin but with much less toxicity may be prepared. The problem of designing and synthesizing an azole nucleoside which will cross the blood brain barrier also remains to be solved.

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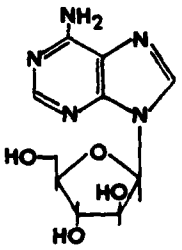
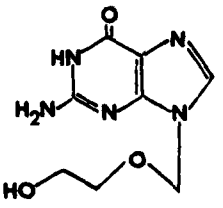
Introduction:

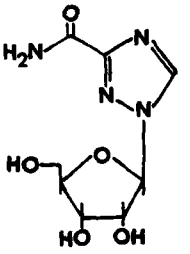
The idea of antiviral agents related to genetic biochemicals was not new in the late 1960s. The antiviral nucleosides like 1-(β-D-arabinofuranosyl)cytosine (ara-C), 5-halogenated derivatives of deoxyuridine, particularly 5-iodo-2'-deoxyuridine (IUdR) and 5-trifluorothymidine, and 9-(β-D-arabinofuranosyl)adenine (ara-A) had been synthesized and studied. Although the development in the area of viral chemotherapy was rather slow, in part because the synthesis of nucleoside analogs was technically difficult and the nucleosides often exhibited toxicity. However, substantial progress has been made in recent years. The FDA approval of ara-A (trade name Vidarabine) for use against herpes infection was indeed a major step forward. The antiviral activity of ara-A has recently been summarized,[1] and even though its use and efficacy would appear to be limited to certain DNA viruses, such as herpes, cytomegalo and vaccinia viruses, and presently must be administered intravenously, it is unquestionably effective against these serious



ara-A



Acyclovir



Ribavirin

and life-threatening diseases. The antiherpetic activity and low toxicity of 9-(2-hydroxyethoxymethyl)guanine (acyclovir) presently appears promising.

A synthetic triazole nucleoside, 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (Ribavirin), which is structurally related to naturally occurring antiviral nucleoside antibiotics pyrazofurin[2] and bredinin[3] was reported by Robins and co-workers in 1972.[4] Ribavirin is a broad spectrum virustatic agent and is important as a clinically useful antiviral drug.[5] In addition to certain DNA viruses, ribavirin is very effective against RNA viruses having substantial implications from a global epidemiologic standpoint (e.g. Rift Valley fever,

Lassa fever, Bolivian hemorrhagic fever and others).[5] The in vitro and in vivo antiviral activity of ribavirin has recently been summarized.[5,6] Since the original report on the synthesis of ribavirin[4] the data generated regarding the understanding of structure-activity relationships, biochemistry and mechanism of action is fascinating.

Ribavirin was found to be clinically effective in ameliorating influenza symptoms and fever, and diminishing the quantity of influenza virus shed by nasal washing.[7] Ribavirin inhibits Chikungunya fever virus in cell culture[8] and is effective in reducing viremia and increasing the number of survivors of Machupo virus infected rhesus monkeys.[9] Treatment of Lassa fever in monkeys with ribavirin gave highly beneficial results.[10,11] Similarly, ribavirin on hamsters inoculated with Pichinde virus gave efficacious results.[9] Ribavirin is active in vitro against yellow fever virus in monkey kidney cells at 100 µg/ml. It is also active in vitro against RVF virus in vero cells at 100-200 µg/ml. The greatest activity of ribavirin in vitro is seen against Sandfly fever in SW13 cells with 100 percent plaque reduction at 20 µg/ml. However, least activity was seen against VEE with only 50 percent plaque reduction at 500 µg/ml concentration of ribavirin. Thus, ribavirin is the only known antiviral agent today which is significantly active against a substantial number of both RNA and DNA viruses. It is also unique in that to date no sensitive rival strain has become resistant to the drug.

Ribavirin is a very specific nucleoside with amazingly stringent structural requirements for broad-spectrum antiviral activity and these structural requirements are viewed as essential to transport the drug into the cells.[12] Presumably the carboxamide group is an essential binding site to the viral RNA polymerase. Ribavirin 5'-phosphate has been shown to be essentially as effective as ribavirin when tested against lethal influenza infections in mice.[13] It also showed good antiviral activity in vitro against yellow fever, Pichinde, VEE and RVF at 100-300 µg/ml. Ribavirin triacetate was essentially inactive in vitro but exhibited in vivo

activity against Machupo virus in monkeys and guinea pigs[9] and against RVF virus in mice. Ribavirin triacetate has also been shown to be superior to ribavirin when used in aerosol form to treat influenza in mice.[14] Ribavirin 2',3'-cyclic phosphate was nearly as active as ribavirin against RVF virus in mice but was inactive against VEE in mice as was ribavirin. In addition to significant antiviral activity in vitro against parainfluenza, herpes and rhino-13 virus,[13] ribavirin 3',5'-cyclic phosphate showed remarkable activity against VEE in vivo.

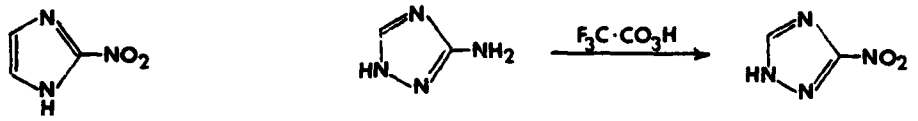
One of the potential problems in the toxicology of ribavirin is a moderate and self-limiting macrocytic, hyperchromic anemia which has been shown to develop in primates.[15] Apparently ribavirin is absorbed into red blood cells where it is phosphorylated to the 5'-phosphate by adenosine kinase and trapped for some time. A ribavirin derivative, such as ribavirin 3',5'-cyclic phosphate which would have different transport characteristics, may not be so readily absorbed by the red blood cells and be highly desirable for clinical studies. Thus, in view of these observations it is quite conceivable that certain ribavirin derivatives may offer significant advantage over the parent nucleoside in drug formulation, drug transport or provide greater organ concentration of the active form of the drug. Therefore, it was proposed that a number of ribavirin derivatives of certain related nucleoside azole carboxamides be prepared and studied for antiviral efficacy.

During the last seventeen months we continued our synthetic program designed to provide the selected ribavirin derivatives and related azole nucleosides. Eighty-one such compounds were prepared and submitted to Medical Research and Development Command, Walter Reed Medical Center, U.S. Army, for antiviral evaluation. The progress made in the synthetic aspect may be divided into three categories.

1. Synthesis of triazole nucleosides related to ribavirin,
2. Synthesis of imidazole carboxamide nucleosides related to ribavirin, and
3. Synthesis of purine nucleoside analogs containing carboxamide function.

1. Synthesis of Triazole Nucleosides Related to Ribavirin

In continuation of our synthetic efforts related to ribavirin and other azole nucleosides, we prepared several 1,2,4-triazole and 1,2,3-triazole ribosides. 3-Nitro-1,2,4-triazole (2) is of considerable interest as it is an aza analog of the naturally occurring antibiotic azomycin. Azomycin, which was isolated[16] from



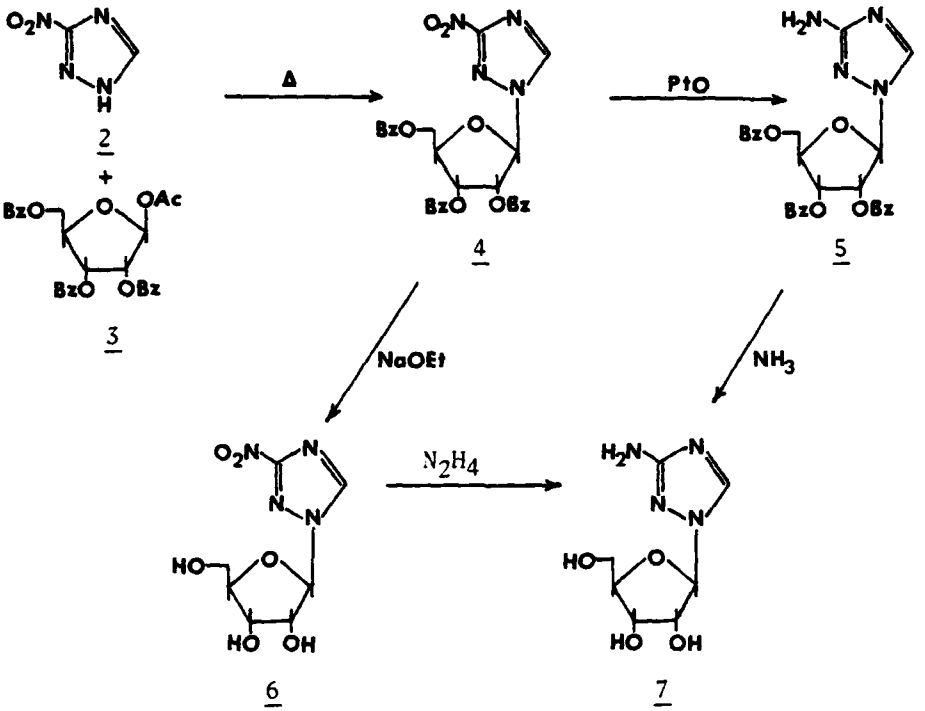
Azomycin 1 2

a strain of Norcordia mesenterica, has been characterized[17] as 2-nitroimidazole and has demonstrated activity against trichomonosis.[18] Studies[19] with [2-^{14}C]2-aminoimidazole indicates that the biosynthesis of azomycin occurs by enzymatic oxidation of the amino group to a nitro group. 2-Nitro-1-(β-D-ribofuranosyl)-imidazole (azomycin riboside) has been prepared by the acid-catalyzed fusion,[20] which exhibited significant antibacterial activity.

Attempts to nitrate 1,2,4-triazole,[21] 3-halogeno-1,2,4-triazoles,[21] and 3-methoxy-1,2,4-triazole[22] have been reported to result only in formation of the nitrate salt or degradation of the starting heterocycle. An alternate procedure for obtaining aromatic nitro compounds is the oxidation of the corresponding amino derivative. Although several reagents have been reported for this purpose, the most generally successful appears to be pertrifluoroacetic acid.[23] Oxidation of 3-amino-1,2,4-triazole (1) at 70-80°C with pertrifluoroacetic acid, prepared from trifluoracetic acid and 90% hydrogen peroxide,[24] readily provided 3-nitro-1,2,4-triazole (2, BJ-91059).[25]

Direct fusion of 2 with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (3) in the absence of a catalyst readily gave an 88% yield of crystalline 3-nitro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazole (4, BJ-91111). No other nucleoside product was detected.[26] When 4 was subjected to hydrogenation

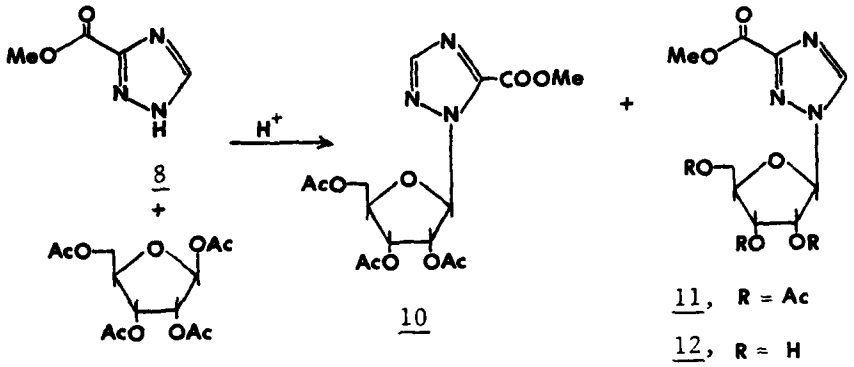
with Pd/C (10%), a mixture of products was obtained even after prolonged reduction.



The major product was separated by fractional crystallization and was established (by pmr, ir, tlc) to be 3-hydroxylamino-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazole.[26] The minor product was found to be 3-amino-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazole (5, BJ-91120). In an effort to improve the yield of 5, compound 4 was hydrogenated in presence of PtO catalyst[27] in tetrahydrofurane at 50 psi for 2 hr. which gave good yield of 5. Debenzoylation of 4 with methanolic sodium methoxide provided 3-nitro-1-(β-D-ribofuranosyl)-1,2,4-triazole (6, BJ-91102).[26] Reduction of the nitro group of 6 with hydrazine gave an 88% yield of 3-amino-1-(β-D-ribofuranosyl)-1,2,4-triazole (7, BJ-91068).[26] Compound 7 was also obtained from 5 by the treatment with methanolic ammonia (methanol saturated with ammonia at 0°C).

The acid-catalyzed fusion of methyl 1,2,4-triazole-3-carboxylate[28] (8) with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (9) in the presence of bis(p-nitrophenyl)-

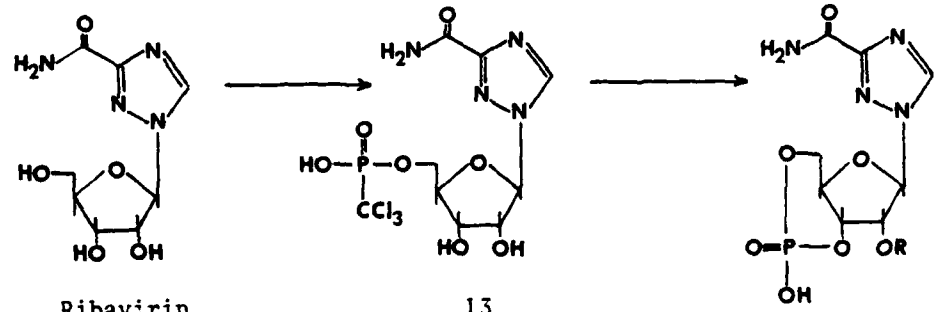
phosphate at 160-165°C provided an 85% yield of methyl 1-(2,3,5-tri-O-acetyl-β-



8 + 9 → 10 + 11 (R = Ac) + 12 (R = H)

-D-ribofuranosyl)-1,2,4-triazole-5-carboxylate (10) and methyl 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (11) in a 1:10 ratio.[4] These positional isomers were separated by column chromatography over silica gel. Deacetylation of 11 with sodium methoxide in methanol gave methyl 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (12, BJ-81517), which was found to be a versatile intermediate for the synthesis of 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylic acid (BJ-58518).

In an effort to improve the yield of ribavirin 3',5'-cyclic phosphate (14, BJ-58536), which exhibited significant antiviral activity against VEE viral infection in mice, ribavirin was reacted with trichloromethylphosphonyl dichloride[29] in anhydrous trimethyl phosphate according to the procedure of Honjo and co-workers[30]

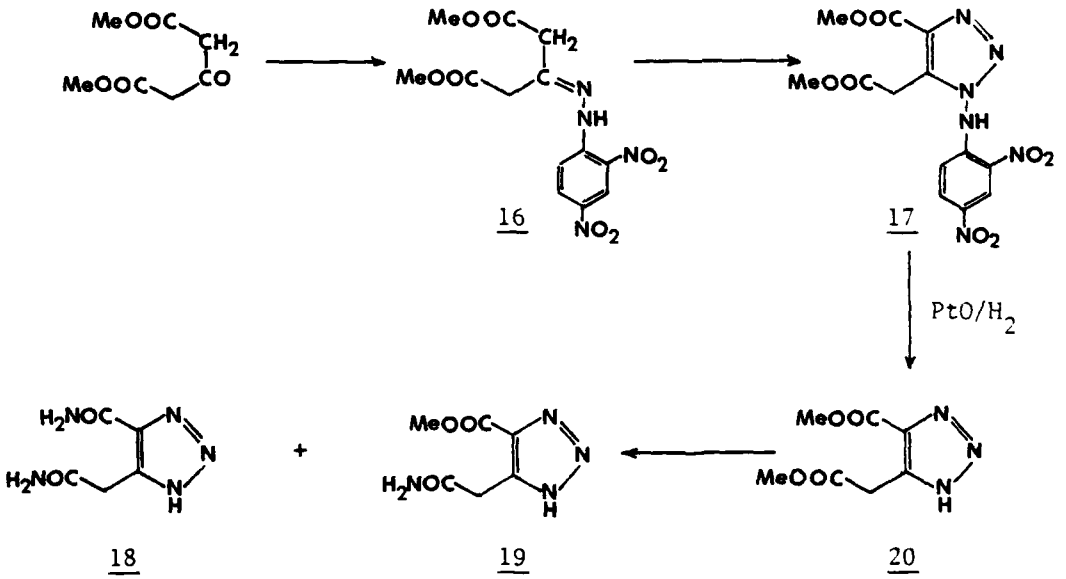


Ribavirin → 13 → 14 (R = H), 15 (R = Ac)

to yield 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide 5'-trichloromethylphosphonate (13, BJ-92529). The base ($KOBu^t$) catalyzed intramolecular cyclization

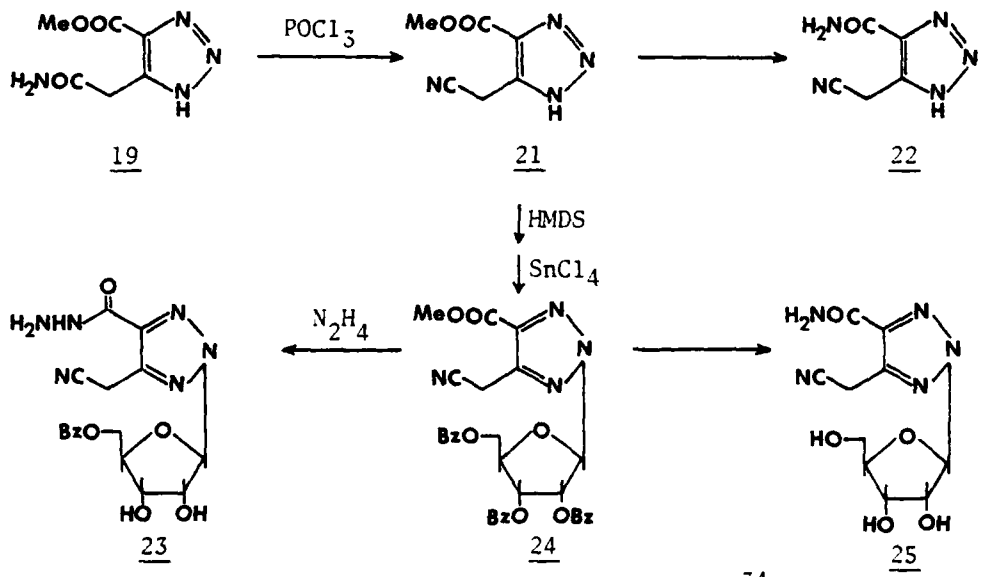
of 13 gave good yield of 14. Acetylation of 14 with acetic anhydride in pyridine at low temperature and extensive resin column chromatography gave 1-(2-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide 3',5'-cyclic phosphate (15, BJ-91095). Compound 15 was prepared in the hope of getting better cellular penetration and possible crossing of the blood brain barrier.

Several appropriately substituted 1,2,3-triazoles and the corresponding nucleosides related to the antiviral agent 5-cyanomethyl-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide (BJ-08465)[31] have been prepared. The synthesis of



methyl 4(5)-methoxycarbonylmethyl-1,2,3-triazole-5(4)-carboxylate (20, BJ-84170), which is amenable to further functional group transformation was pursued by novel ring closure method. Under the catalysis of 1,5-diazabicyclo[5.4.0]-undec-5-ene (DBU), the 2,4-dinitrophenylhydrazone of dimethyl 1,3-acetonedicarboxylate (16) was converted to methyl 5-methoxycarbonylmethyl-1-(2,4-dinitroanilino)-1,2,3-triazole-4-carboxylate (17) with p-toluenesulfonyl azide in 90% yield. This cyclization is analogous to the reported conversion of α-diazoimines, generated oxidatively from 1,2-bishydrazones, to 1-amino-1,2,3-triazoles.[32] Catalytic reduction of 17 in the presence of platinum oxide readily gave 20 in over 82%

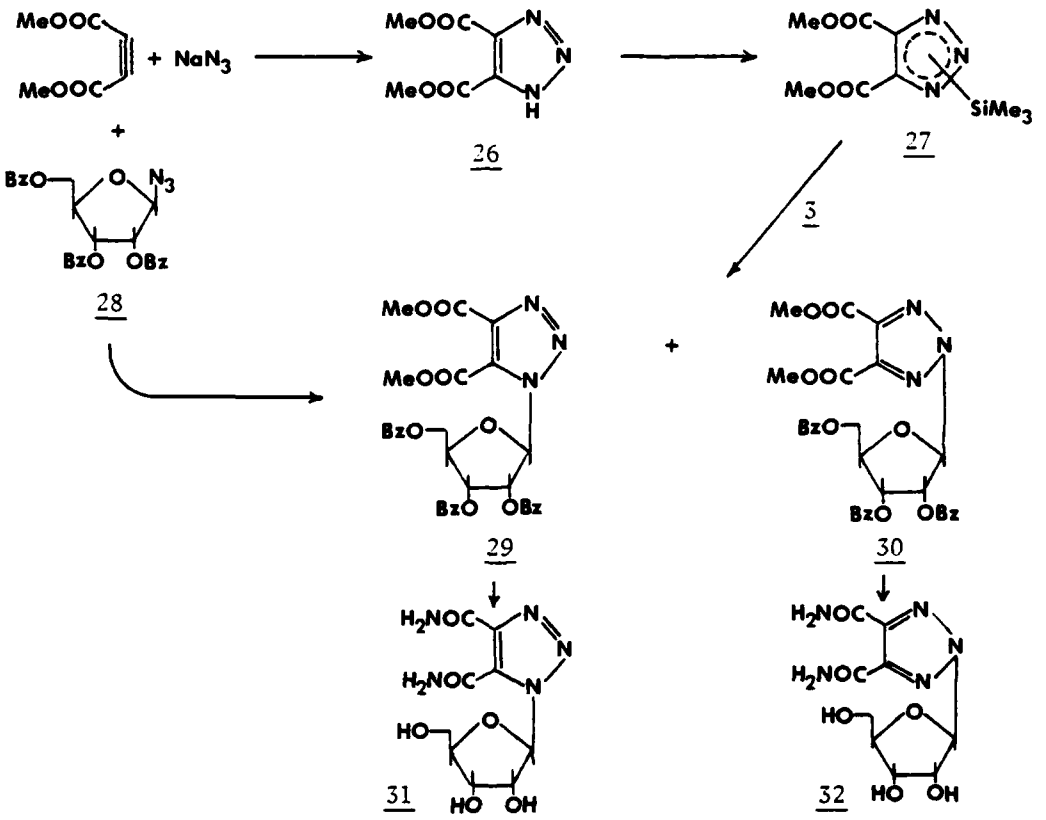
yield. The pmr spectrum is in support of this structure. Ammonolysis of 20 with concentrated NH_4OH at room temperature gave, in addition to the expected[33] methyl 4(5)-carbamoylmethyl-1,2,3-triazole-5(4)-carboxylate (19), the diamide 4(5)-carbamoylmethyl-1,2,3-triazole-5(4)-carboxamide (18). However, this reaction appears to be temperature dependent, since treatment of 20 with cold (0-5°C) concentrated NH_4OH gave exclusively 19. The mixture of 18 and 19 were separated by fractional crystallization; the less soluble (in ethanol) diamide 18 crystallized first. Dehydration of 19 with boiling $POCl_3$ gave good yield of methyl



19 21 22 23 24 25

4(5)-cyanomethyl-1,2,3-triazole-5(4)-carboxylate (21).[34] The structure of 21 was confirmed by observing a nitrile and ester absorption band at 2260 and 1715 cm^{-1}, respectively, in the ir spectrum. Treatment of 21 with liquid NH_3 at 100-110°C for 10 hr. provided 4(5)-cyanomethyl-1,2,3-triazole-5(4)-carboxamide (22, BJ-84189).[34] The ir spectrum of 22 also possessed a nitrile band at 2220 cm^{-1} and an amide carbonyl band at 1655 cm^{-1}, in place of the ester carbonyl band of 21. Stannic chloride (0.72 molar equivalent) catalyzed glycosylation of the trimethylsilyl derivative of 21 (1.0 molar equivalent) with 3 (1.0 molar equivalent) in 1,2-dichloroethane at room temperature gave 94% yield of crystalline methyl 5-cyanomethyl-2-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,3-triazole-4-carboxylate (24, BJ-84198),

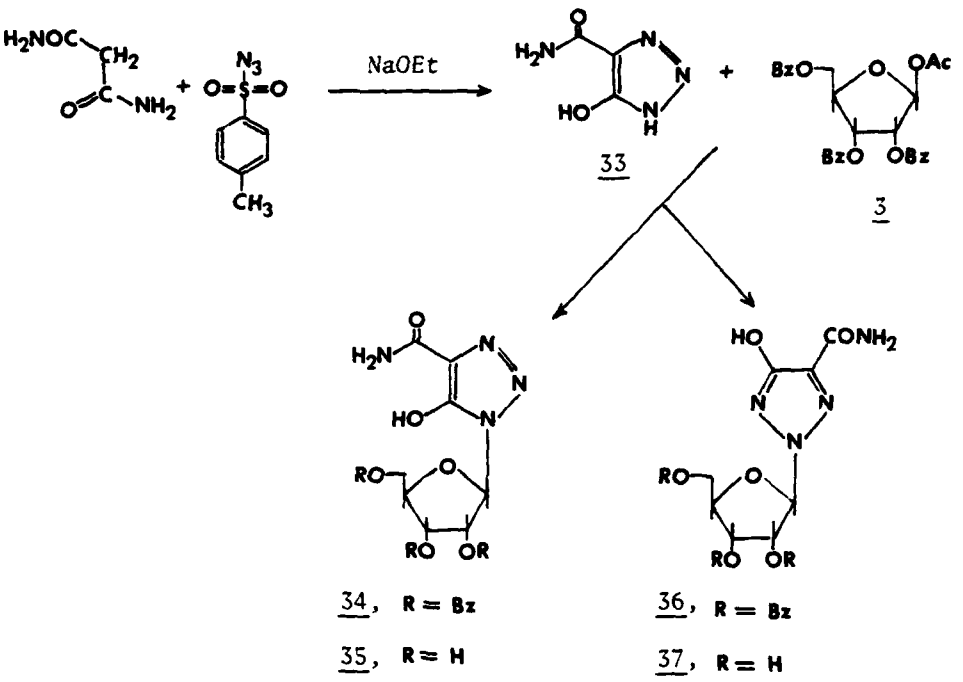
along with minor amounts of the positional isomers.[34] Debenzoylation of 24 with concomitant amination of the ester function was achieved in good yield by the treatment with methanolic ammonia at room temperature to obtain crystalline 5-cyanomethyl-2-(β-D-ribofuranosyl)-1,2,3-triazole-4-carboxamide (25, BJ-84205).[34] Partial debenzoylation occurred when 24 was treated with methanolic hydrazine at room temperature to yield crystalline 5-cyanomethyl-2-(5-O-benzoyl-β-D-ribofuranosyl)-1,2,3-triazole-4-carboxhydrazide (23). Compound 24 exhibited significant antiviral activity against RVFV in vitro. In view of this activity, we prepared several other 1,2,3-triazole nucleosides structurally related to 24.



For the synthesis of such compounds 4,5-dicarbomethoxy-1,2,3-triazole (26, BJ-92449) was proved to be a versatile starting material. Addition of an azide ion to activated alkynes in an aprotic solvent (like DMF) is reported to yield 1,2,3-triazoles.[35] Thus treatment of freshly distilled dimethyl acetylenedicar-

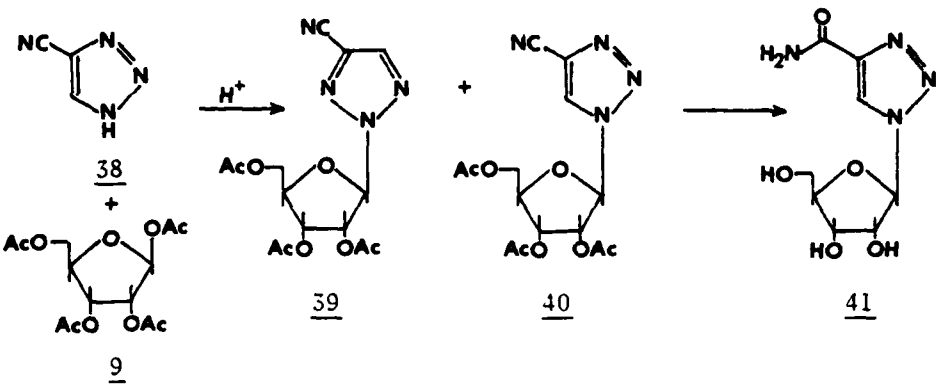
boxylate to a suspension of sodium azide in DMF readily gave 26 in 60% yield.[36] Direct glycosylation of the trimethylsilyl derivative of 26 (27) with 3 in the presence of a novel Lewis acid catalyst trimethylsilyl trifluoromethanesulfonate ($F_3C \cdot SO_2 \cdot OSiMe_3$) gave a mixture of two isomeric dimethyl 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,3-triazole-4,5-dicarboxylate (29, BJ-92485) and the 2-glycosyl isomer (30). These isomers were separated by silica gel column chromatography using chloroform-benzene (1:1) as eluant. The isomeric ratio being approximately 4:1, in favor of 30. In an effort to improve the yield of 29, dimethyl acetylene-dicarboxylate was reacted with 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl azide (28)[37] in boiling benzene to obtain exclusively 29, according to the procedure of Baddily and co-workers.[38] Deacetylation of 29 and 30 with methanolic ammonia gave 1-(β-D-ribofuranosyl)-1,2,3-triazole-4,5-dicarboxamide (31, BJ-92494) and 2-(β-D-ribofuranosyl)-1,2,3-triazole-4,5-dicarboxamide (32, BJ-92458), respectively.

The C-nucleoside antibiotic pyrazofurin is of particular interest because of its broad spectrum antiviral activity in vitro. However, its practical utility as an antiviral drug is in question because of its profound toxicity in mice.[39] Pyrazofurin could not be administered at doses greater than 1 mg/kg/day. As the toxicity in mice may be associated with structural features that are not necessarily related to the antiviral potency of this nucleoside, it should be possible to alter the structure of pyrazofurin in such a way as to decrease its toxic properties and yet retain the potent antiviral activity. One such structural modification in which we were interested, is to introduce a nitrogen at the glycosidic linkage to provide an antimetabolite N-nucleoside analog of pyrazofurin (35). The logical starting material for the chemical synthesis of 35 is the heterocycle 4(5)-hydroxy-1,2,3-triazole-5(4)-carboxamide (33), which on glycosylation should provide the desired 35. A large amount of 33 (BJ-86325) has been prepared by condensing malondiamide with p-toluenesulfonyl azide in the presence of sodium



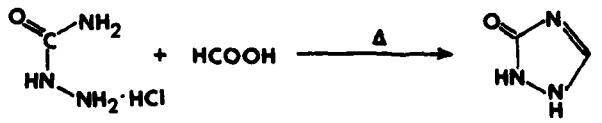
ethoxide according to the procedure of Dimroth.[40] Glycosylation of the trimethyl silyl derivative of 33 with 3 in the presence of either $SnCl_4$ or $F_3C{\cdot}SO_2{\cdot}OSiMe_3$ gave a mixture of two isomeric protected nucleosides, 34 and 36, which were separated by silica gel column chromatography using $CHCl_3$:MeOH (9:1) as eluant. Debenzoylation of pure blocked anomers with $NaOCH_3$ in MeOH at ambient temperature provided 5-hydroxy-1-(β-D-ribofuranosyl)1,2,3-triazole-4-carboxamide (35) and the corresponding N_2-glycosyl isomer (37, BJ-92547).

Fusion of 4-cyano-1,2,3-triazole (38)[41] with 9 in the presence of bis(p-nitrophenyl)phosphate provided[42] two protected nucleosides, 4-cyano-2-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,3-triazole (39) and 4-cyano-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,3-triazole (40), in almost 1:1 ratio, which were separated by silica gel column chromatography. Treatment of 40 with 30% hydrogen peroxide in an ammoniacal solution readily gave 1-(β-D-ribofuranosyl)-1,2,3-triazole-4-carboxamide (41, BJ-81535).[42]



38 + 9 → 39 + 40 → 41

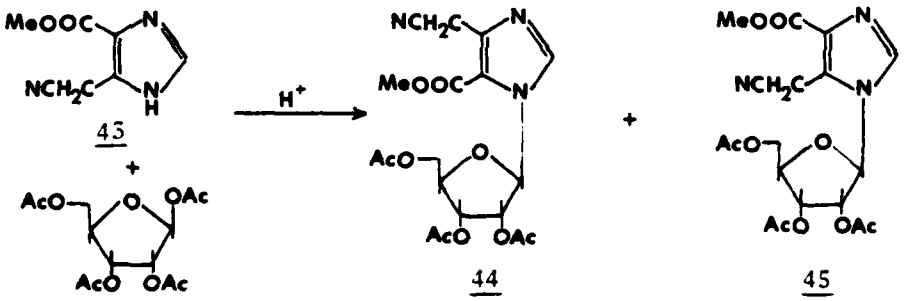
A simple derivative of 1,2,4-triazole ring system, 1,2,4-triazolin-3-one (42) has been prepared[43] by the ring-annulation of semicarbazide hydrochloride with formic acid at 100°C. Compound 42 may be considered as dehydrourazole.



42

2. Synthesis of Imidazole Carboxamide Nucleosides Related to Ribavirin

In the imidazole carboxamide nucleoside series, the preparation of methyl 4-cyanomethyl-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylate (44, BJ-91040) was accomplished by fusion of methyl 5(4)-cyanomethylimidazole-

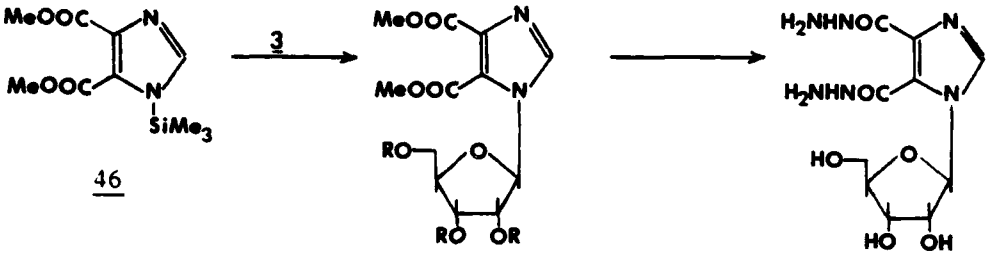


43 + 9 → 44 + α-isomer + 45 + α-isomer

4(5)-carboxylate (43)[44] with 9 at 155°C in the presence of acid catalyst bis(p-nitrophenyl)phosphate, which provided a complex reaction mixture of four positional

and isomeric nucleosides. After silica gel column chromatography, compound 44 was isolated in pure form as white needles.[44]

When dimethyl 1-trimethylsilylimidazole-4,5-dicarboxylate (46), obtained by the silylation of dimethylimidazole-4,5-dicarboxylate with hexamethyldisilazane, was treated with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (3) in the presence of 1.44 molar equivalent of anhydrous $SnCl_4$ afforded dimethyl 1-(2,3,5-



46

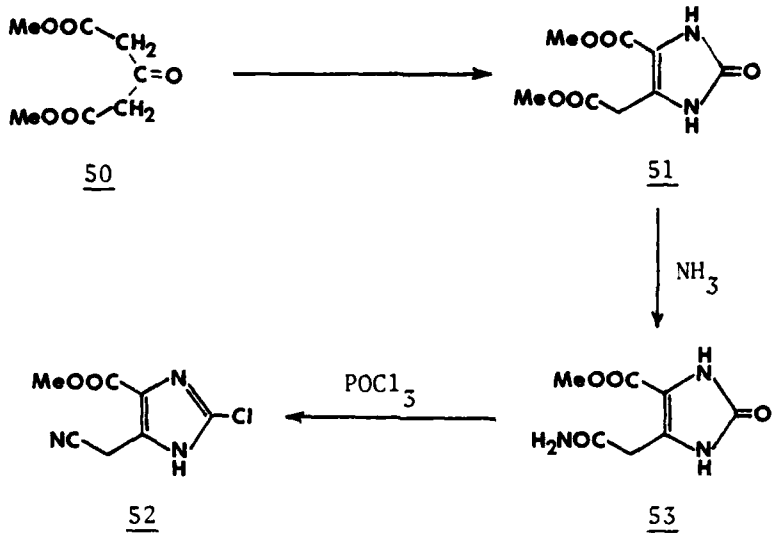
47, R = Bz

48, R = H

49

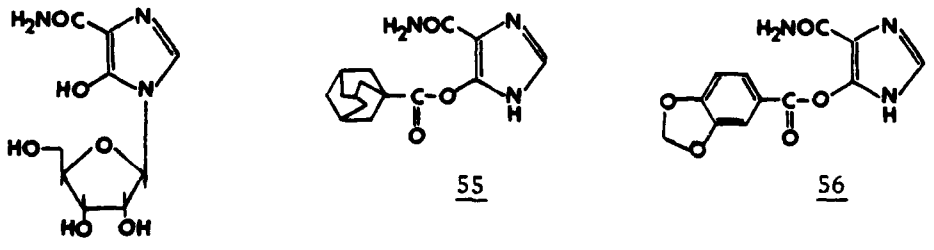
tri-O-benzoyl-β-D-ribofuranosyl)imidazole-4,5-dicarboxylate (47, BJ-84161) in 90% yield.[45] Debenzoylation of 47 with sodium methoxide in methanol readily gave dimethyl 1-(β-D-ribofuranosyl)imidazole 4,5-dicarboxylate (48, BJ-81553). The protected nucleoside 47, or the deblocked nucleoside 48, was converted into 1-(β-D-ribofuranosyl)imidazole-4,5-carboxyhydrazide (49) in 94% yield with refluxing ethanolic hydrazine hydrate.[45]

Several imidazole derivatives were also prepared during the course of these synthetic studies. The preparation of methyl 2-chloro-5-cyanomethyl-4-carboxylate (52) was undertaken. Compound 52 is a versatile starting material for novel purine base analog 8-chloro-3-deazaguanine. The commercial availability of methyl acetonedicarboxylate (50) provided a ready source of starting material for the synthesis of 52. Nitrosation of 50 with sodium nitrite in glacial acetic acid gave dimethyl-2-nitroso-3-keto-glutarate which, on reduction with sodium

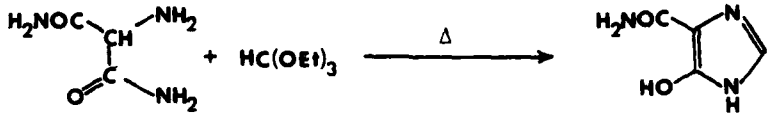


hydrosulfite followed by ring-annulation with potassium cyanate in the presence of sulfuric acid gave 5-acetic acid-2-imidazolone-4-carboxylic acid dimethyl ester (51).[33] When 51 was treated with aqueous ammonia on a steam bath, a monoamide, methyl 5-acetamide-2-imidazolone-4-carboxylate (53) was readily obtained. Treatment of 53 with $POCl_3$ at reflux temperature gave methyl 2-chloro-5-cyanomethyl-imidazole-4-carboxylate (52).[33] Compound 52 will be subsequently ring closed with ammonia to 8-chloro-3-deazaguanine. It is of particular interest that 3-deazaguanine demonstrated a potent broad spectrum activity against various RNA and DNA viruses in vitro.[31,44]

Bredinin (54) is a naturally occurring nucleoside antibiotic reported by Mizuno et al. in 1974,[46] having antiviral properties to inhibit the growth of

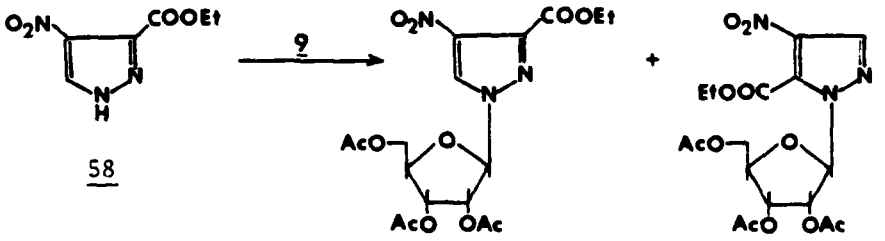


vaccinia virus. Two of the bredinin base derivatives, 4-carbamoylimidazol-4-yl-1-admantanecarboxylate (55) and 4-carbamoylimidazol-4-yl piperonylate (56) were also found to exhibit potent antiviral and antitumor activity.[46] Since 5-hydroxyimidazole-4-carboxamide (57) is the basic aglycon moiety for 54, 55 and 56, the synthesis and antiviral evaluation of 57 is of particular interest. Compound 57 (BJ-92510) was obtained when aminomalonamide was treated with an excess of ethyl orthoformate at 145°C.[47]



57

Glycosylation of ethyl 4-nitropyrazole-3-carboxylate (58) with 9 by the non-catalytic fusion procedure as described by Preobrazhenskaya et al.[48] gave a mixture of ethyl 4-nitro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-3-carboyxl[illegible] (59) and the positional isomer ethyl 4-nitro-1-2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-5-carboxylate (60, BJ-76267).[48] The mixture of these two isomers were separated on a silica gel column using $CHCl_3$:MeOH (19:1) as the solvent. The N-2 glycosyl isomer (60) was eluted first.



58

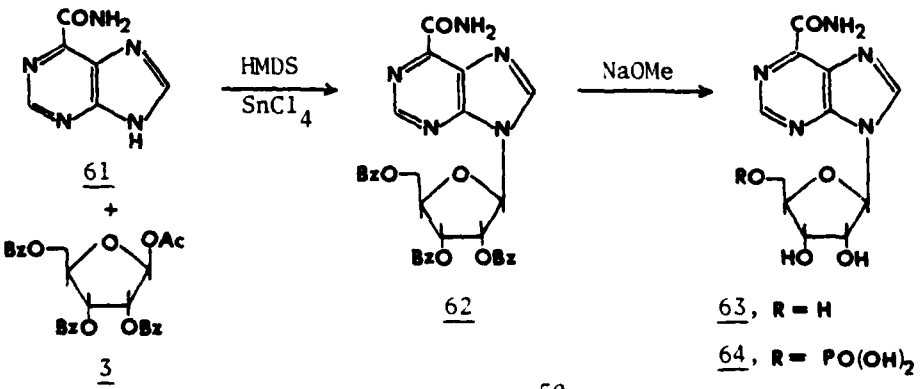
59

60

3. Synthesis of Purine Nucleoside Analogs Containing Carboxamide Function

One of the most interesting features of ribavirin is the presence of a hydrogen bonding carboxamide function, which is absolutely essential for its broad

spectrum antiviral activity.[12] Indeed, the carboxamide function is an integral part of several naturally occurring nucleoside antibiotics such as pyrazofurin, bredinin and sangivamycin. A major disadvantage of ara-A is its relative insolubility in water, which makes its intravenous administration difficult.[49] The introduction of a craboxamide group due to its polar properties should make the nucleoside readily soluble in water. It could also facilitate the binding of the nucleoside with proteins and nucleic acids which contain such a functionality (-CONH-) as an inherent part of the molecule. To examine these structural parameters for antiviral potency, we recently prepared 9-(β-D-ribofuranosyl)purine-6-carboxamide (BJ-42341), which exhibited potent antiviral activity at nontoxic dosage levels against a number of RNA and DNA viruses in cell culture. BJ-42341 employed in the treatment of RVF virus infected mice at 50 mg/kg/day gave a 55% survival rate on day 21 compared to a 30% survival in the controls. Ribavirin, however, at 100 mg/kg/day in the same experiment, gave a 70% survival rate on day 21. The chemical synthesis of 9-(β-D-ribofuranosyl)purine-6-carboxamide (63) was accomplished by two different routes.

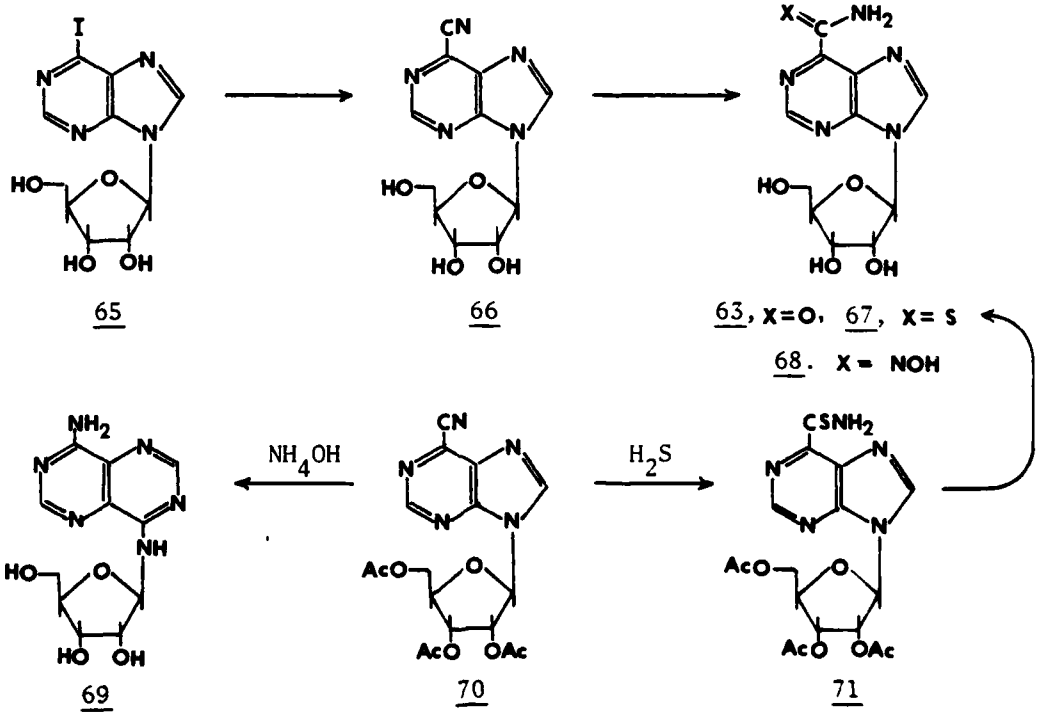


61 + 3 → 62 → 63, R = H; 64, R = PO(OH)$_2$

Treatment of purine-6-carboxamide (61)[50] with hexamethyldisilazane gave the TMS derivative which was then coupled with one equivalent of 3 in presence of 1.4 molar equivalent of $SnCl_4$ in dichloroethane at ambient temperature. Under these conditions, an 83% yield of 9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)purine-6-carboxamide (62) was obtained. Careful investigation of the mother liquor

furnished chromatographic evidence of the formation of other nucleoside material in very minor amount, presumably the positional isomer. Debenzoylation of 62 with MeOH/NaOMe gave the desired 63. Direct phosphorylation[51] of unprotected 63 with $POCl_3$ in trimethylphosphate at 0-5°C, followed by hydrolysis, gave 9-(β-D-ribofuranosyl)purine-6-carboxamide 5'-phosphate (64, BJ-81544). The purity and structure of 63 and 64 was confirmed by elemental and pmr analyses.

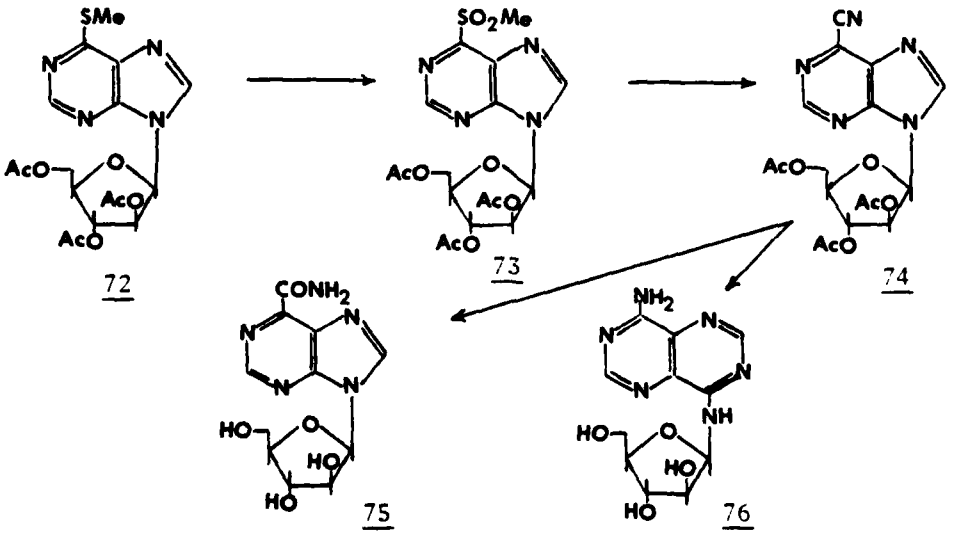
The synthesis of 63 was also approached by an alternate route for which 9-(β-D-ribofuranosyl)purine-6-carbonitrile (66, BJ-63939) was found to be a



versatile intermediate. When 9-(β-D-ribofuranosyl)-6-iodopurine (65, BJ-63920)[52] was treated with CuCN in pyridine at reflux temperature, a 60% yield of crystalline 66 was obtained, which was found to be stable and the ir spectrum revealed a weak nitrile band at 2240 cm^{-1}. Treatment of 66 with cold H_2O_2 in alkaline media (pH 8.5) gave 63. When 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine-6-carbonitrile (70) which was obtained[53] by the acid catalyzed fusion of 6-cyanopurine and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose, was treated with H_2S in pyridine 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine-6-thiocarboxamide (71, BJ-76221) was obtained.

Deacetylation of 71 furnished 9-(β-D-ribofuranosyl)purine-6-thiocarboxamide (67, BJ-81526). The pmr spectrum of 67 revealed the two thiocarboxamide resonances as more downfield (δ 10.1 and 10.5) compared to the carboxamide resonances (δ 8.06 and 8.38) of 63. When 66 was allowed to react with free NH_2OH in EtOH, nearly quantitative yield of 9-(β-D-ribofuranosyl)purine-6-carboxamidoxime (68, BJ-63902) was obtained. The ir spectrum of 71, 67 and 68 did not show any absorption at 2220 cm^{-1} region. Treatment of 70 with a large excess of NH_4OH at room temperature gave the rearrangement product 4-amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-d]pyrimidine (69, BJ-76187),[53] the structure of which has recently been confirmed by x-ray crystallographic studies.[54]

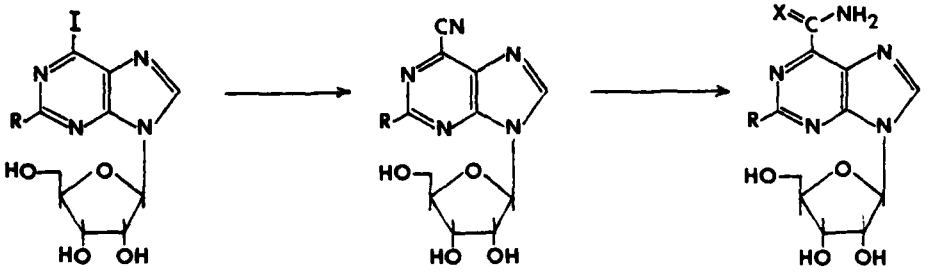
In view of the potent antiviral activity and increased water solubility of purine-6-carboxamide riboside (63, BJ-42341) we undertook the synthesis of 9-(β-D-arabinofuranosyl)purine-6-carboxamide (75) and certain related compounds for which 9-(2,3,5-tri-O-acetyl-β-D-arabinofuranosyl)purine-6-carbonitrile (74) served as a versatile intermediate. Acetylation of 6-methylthio-9-(β-D-arabinofuranosyl)purine[55] with acetic anhydride using a catalytic amount of 4-(N,N'-



dimethylamino)pyridine provided 6-methylthio-9-(2,3,5-tri-O-acetyl-β-D-arabinofuranosyl)purine (72) as a chromatographically homogeneous syrup in 90% yield.

The $KMnO_4$ oxidation of 72 in acetic acid provided 6-methylsulfonyl-9-(2,3,5-tri-O-acetyl-β-D-arabinofuranosyl)purine (73) as a syrup in 80% yield. In the pmr spectrum ($CDCl_3$) of 73, all the protons, except acetyls and C_4,H and C_5,H_2, were shifted downfield as compared to those of 72. The SCH_3 protons had a shift of 0.75 ppm, whereas C_2H, C_8H and H_1, were shifted by 0.42, 0.47 and 0.13 ppm, respectively. The downfield shift would be expected due to the sulfonyl group in 73. Treatment of NaCN with 73 in DMF provided the key intermediate 74 in about 50% yield after extensive column chromatographic purification. Attempts to crystallize this syrupy product were unsuccessful. It did not exhibit significant absorption in the ir for the nitrile band, however was fully characterized by physical and chemical methods. Treatment of 74 with NH_4OH in the presence of H_2O_2 under carefully controlled conditions (like pH and temperature) provided the desired 9-(β-D-arabinofuranosyl)purine-6-carboxamide (75, BJ-91086) as water-soluble needles in 50% yield.[56] The structure of 75 was established on the basis of pmr which exhibited, in addition to other protons at appropriate positions, the peaks at δ 8.05 and 8.38 as broad singlets for $CONH_2$ protons which were exchanged by D_2O. When 74 was treated with large excess of methanolic ammonia, the rearranged product 4-amino-8-(β-D-arabinofuranosylamino)pyrimido[5,4-d]pyrimidine (76) was obtained.[56] Compound 76 was characterized by pmr, in which the 8-NH proton was coupled with anomeric proton, appeared as a doublet (J = 9.3 Hz) at δ 7.8. The anomeric proton which was further coupled with C_2,H appeared as a pair of doublets ($J_{H_1',-H_2'}$ = 4.0 Hz, $J_{H_1',-NH}$ = 9.3 Hz) centered at δ 6.0. The exchange of active hydrogens by D_2O or spin decoupling of the signal centered at δ 7.80 caused collapse of the pair of doublets at δ 6.0 to a doublet ($J_{H_1',-H_2'}$ = 4.0 Hz) at δ 6.0. The uv spectrum was very similar to that observed for 69. The structure was further substantiated by elemental analysis.

Studies were extended to provide several 2-substituted-9-(β-D-ribofuranosyl)-purine-6-carboxamide derivatives. Treatment of 2-amino-9-(β-D-ribofuranosyl)-6-



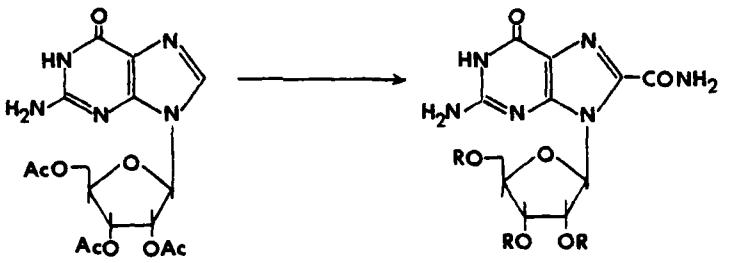
77 78 79, X = O 80, X = S

Series a, R = NH_2

b, R = CH_3

iodopurine (77a, BJ-63957), which was obtained from 2-amino-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-chloropurine (BJ-76196) via 2-amino-9-(β-D-ribofuranosyl)-6-chloropurine (BJ-63948), with CuCN in boiling pyridine gave 2-amino-9-(β-D-ribofuranosyl)purine-6-carbonitrile (78a, BJ-63966). Reaction of 78a with alkaline H_2O_2 or H_2S in pyridine readily gave 2-amino-9-(β-D-ribofuranosyl)purine-6-carboxamide (79a) and the corresponding thiocarboxamide (80a), respectively. Similar reactions starting with 2-methyl-9-(β-D-ribofuranosyl)-6-iodopurine (77b, BJ-76230), which in turn was obtained from 2-methyl-9-(β-D-ribofuranosyl)-6-chloropurine (BJ-76203)[57] led to the isolation of 2-methyl-9-(β-D-ribofuranosyl)-6-cyanopurine (78b, BJ-76249) and 2-methyl-9-(β-D-ribofuranosyl)purine-6-thiocarboxamide (80b, BJ-76258).

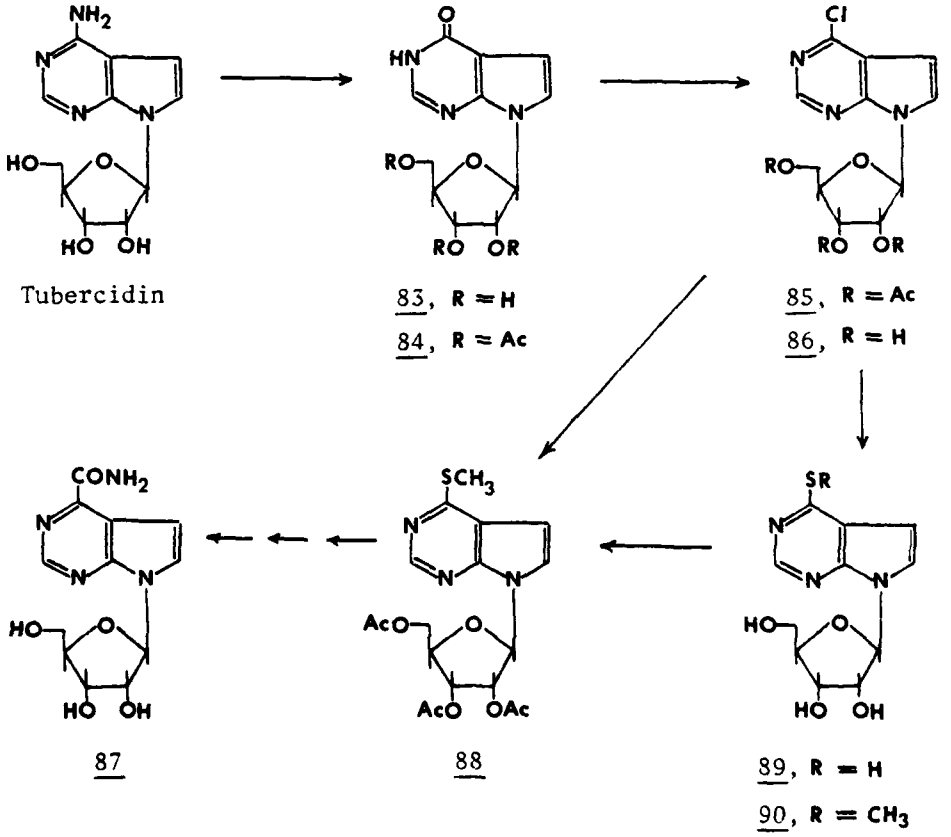
Homolytic acylation[58] of 2',3',5'-tri-O-acetylguanosine with formamide in cold 3N H_2SO_4 using ammonium persulfate as free redical source, gave good yield of fluoroscent 2',3',5'-tri-O-acetyl-8-carbamoylguanosine (81, BJ-76212). Deacetylation of 81 with methanolic NH_3 gave 8-carbamoylguanosine (82). The structure of 82 was assigned by the pmr spectrum, which showed the expected new peaks for the $CONH_2$ function in addition to the loss of the C_8-proton.



81, R = Ac

82, R = H

We further extended this work to provide new and novel purine nucleoside analogs containing the carboxamide function which may bind to the viral enzyme required for viral replication. One of the first such compounds made was in the tubercidin series. The starting material required for the synthesis of 7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-carboxamide (87) was 4-methylthio-7-



Tubercidin

83, R = H

84, R = Ac

85, R = Ac

86, R = H

87

88

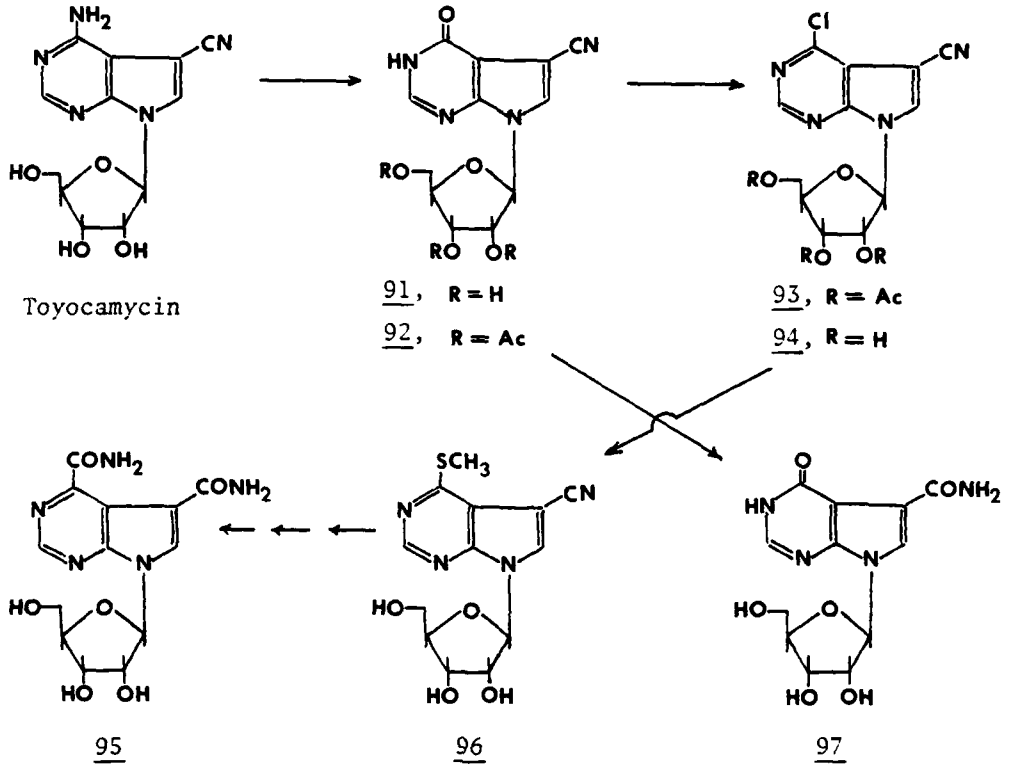
89, R = H

90, R = CH_3

(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (90), which was prepared from tubercidin. Deamination of tubercidin with aqueous nitrous acid at 70-80°C for an hour provided a 90% yield of 7-deazainosine (83, BJ-84125).[59] Acetylation of 83 with a mixture of acetic anhydride and pyridine furnished a good yield of 7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one (84, BJ-84134). Chlorination of 84 with $POCl_3$ at 70-75°C for 30 min. readily gave a 70% yield of 4-chloro-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (85). Removal of the protecting groups on the carbohydrate moiety with cold methanolic ammonia furnished the intermediate 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (86, BJ-84143).[60] Treatment of 86 with thiourea in an aqueous media produced 7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-thione (89, BJ-84152) which, on subsequent alkylation with methyl iodide in sodium methoxide gave 90 (BJ-63886) in good yield.[60] Acetylation of 90 with acetic anhydride in pyridine gave 4-methylthio-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (88).

The 4-chloro substituent of 85 was found to be very susceptible to nucleophilic displacement and provided another pathway requiring less steps and time to the nucleoside 88, rather than going through 90. Thus direct nucleophilic displacement of the 4-chloro group in 85 by the treatment with methylmercaptan in the presence of stoichiometric amount of potassium t-butoxide gave 88 in good yield. In a preliminary small scale run, the $KMnO_4$ oxidation of 88 in acetic acid gave the corresponding 6-methylsulfonyl derivative, which with NaCN in DMF gave 4-cyano-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine. Subsequent treatment of the cyano compound with NH_4OH and H_2O_2 under controlled conditions provided the desired 7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-4-carboxamide (87). Large scale preparation of 87 for antiviral evaluation is in progress.

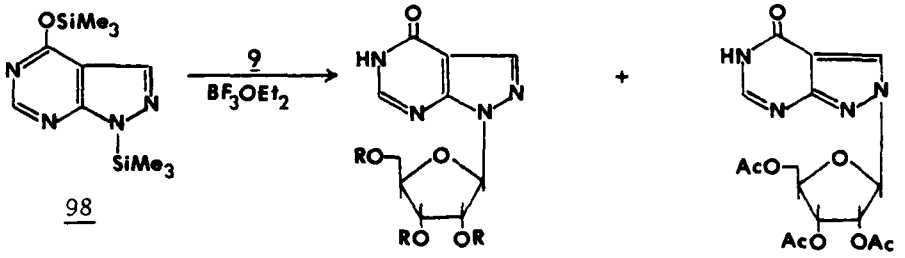
Nucleosides containing two carbamoyl functions such as 95 are readily obtainable from toyocamycin. Deamination of the exocyclic amino group of toyocamycin was accomplished with nitrous acid to afford 5-cyano-7-(β-D-ribofuranosyl)pyrrolo-



[2,3-d]pyrimidin-4-one (91, BJ-63895).[61] Treatment of 91 with acetic anhydride in pyridine gave 2',3',5'-tri-O-acetylated product (92, BJ-86307). The sangivamycin analog, 5-carbamoyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one (97) was prepared by the treatment of 91 with H_2O_2 under alkaline conditions.[61] Chlorination of 92 with $POCl_3$ at reflux temperature for 5 min. furnished good yield of 4-chloro-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (93) which, on deacetylation with cold saturated methanolic ammonia, gave 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (94, BJ-76276) in 68% yield. Treatment of 94 with $KSCH_3$ in ethanol gave 4-methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (96, BJ-76294).[62] Introduction of the 4-cyano function via the methylsulfone derivative of 96 should give the 4,5-dicyano compound, which could be subsequently hydrolyzed with NH_4OH/H_2O_2 to the bis-carboxamide derivative, 95.

Since the pyrazolo[3,4-d]pyrimidine ring is isomeric with purine, the derivatives of pyrazolo[3,4-d]pyrimidine exhibit many properties very similar to those of the corresponding purines. The hypoxanthine analog, pyrazolo[3,4-d]-pyrimidin-4-one (allopurinol) was first reported by Prof. Robins in 1956.[63] Currently it is the drug of choice for the treatment of gouty arthritis. Both allopurinol and its ribonucleoside are effective in preventing the transformation of the intracellular form of L. donovani to the extracellular promastagote form.[64] In view of this data and the potent antiviral activity noted for 9-(β-D-ribofuranosyl)purine-6-carboxamide, we initiated the synthesis of 1-(β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine-4-carboxamide (105).

The condensation of N^1,O^4-bistrimethylsilylpyrazolo[3,4-d]pyrimidin-4-one (98) with tetraacetylribose (9) in dioxane in the presence of borontrifluoride diethyletherate at reflux temperature gave 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidin-4-one (99, BJ-84090) and the corresponding N_2-glycosyl



98

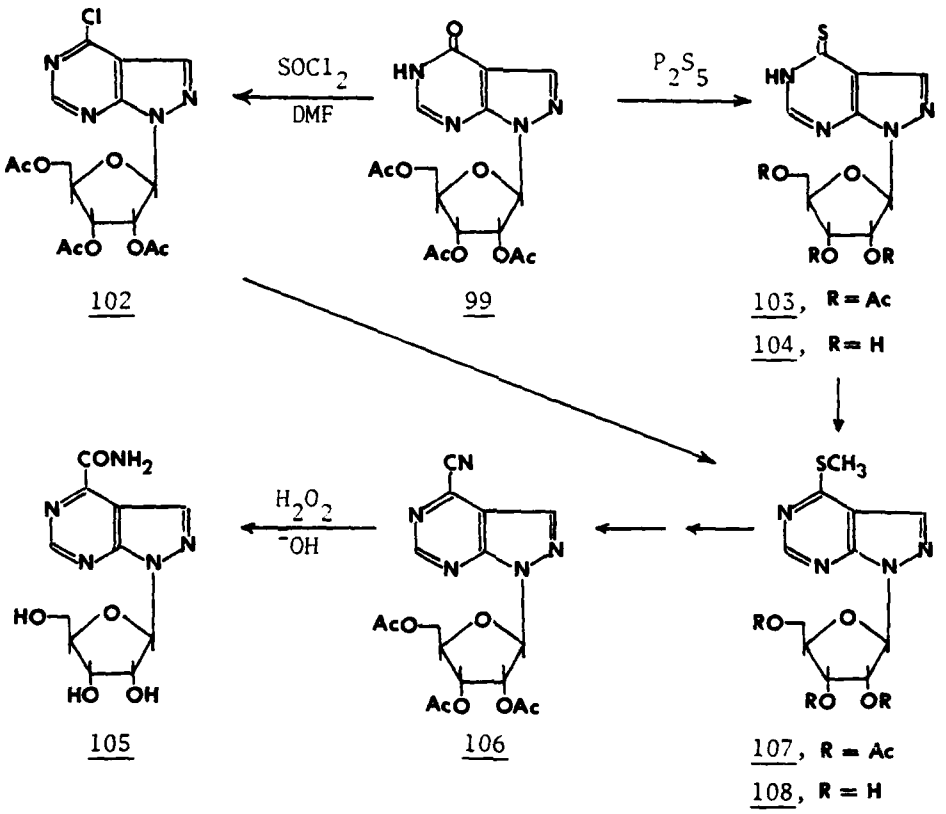
99, R = Ac

100, R = H

101

isomer (101).[65] The mixture of 99 and 101 was separated by silica gel column chromatography using chloroform:acetone (9:1) as the solvent. Deacetylation of 99 with MeOH/NH_3 (saturated at 0°C) afforded allopurinol riboside (100, BJ-86290).

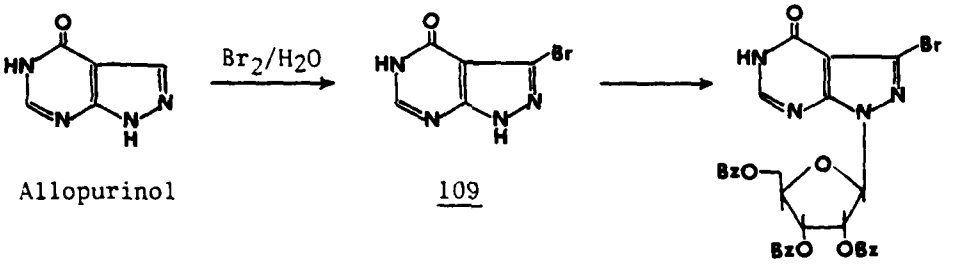
Thiation of 99 with purified P_2S_5 in dioxane provided 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidin-4-thione (103, BJ-86316) in 90% yield.[66] The acetyl groups on 103 were removed with methanolic ammonia to furnish 1-(β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidin-4-thione (104, BJ-63868), a new isomer of



6-mercaptopurine riboside. Treatment of 104 with methyl iodide under alkaline reaction conditions gave 4-methylthio-1-(β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (108, BJ-63877).[66] Chlorination of 99 using mild chlorinating agent dimethyl-chloromethyleneammonium chloride ($SOCl_2$/DMF) afforded 4-chloro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (102, BJ-84107). The 4-chloro group of 102 was found to be amenable to nucleophilic displacement and provided a route for the preparation of 4-methylthio-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (107). When 102 was allowed to react with potassium methylthiolate under controlled pH conditions, 107 (BJ-84116) was obtained in good yield. Compound 107 was proved to be a very useful intermediate for the synthesis of 105. In a preliminary set of reactions, the oxidation of 107 using m-chloroperoxybenzoic acid in anhydrous ethyl ether or chloroform provided the crude oxidation product, the methylsulfone, which was found to be

very susceptible to acidic/alkaline hydrolysis. Therefore without purification, the methylsulfone intermediate was reacted with NaCN in DMF to provide the desired 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidin-4-carbonitrile (106). Treatment of 106 with H_2O_2 in the presence of NH_4OH should provide the desired 1-(β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine-4-carboxamide (105).

There is a scant mention of 3-substituted derivatives of allopurinol or other inosine analogs in the literature. In view of potent in vitro antiviral activity of certain inosine analogs[67] like formycin B, 7-deazainosine and allopurinol riboside against PICH virus, the synthesis of 3-bromoallopurinol (109) and the corresponding riboside was undertaken, in which the 3-bromo function is amenable to further functional group transformations. Prolonged treatment of allopurinol with bromine water under reflux gave the 3-bromo deriv-



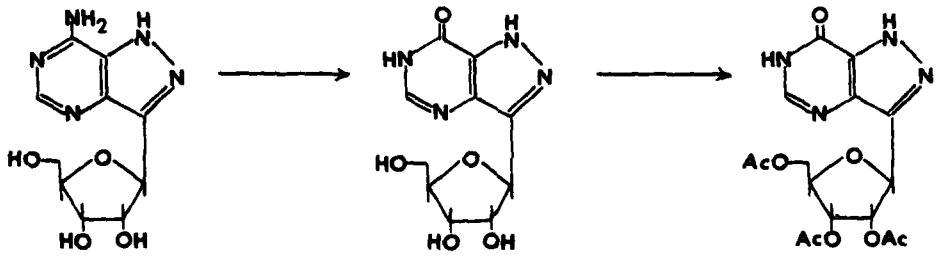
Allopurinol 109 110

ative 109.[68] The N^1,O^4-bistrimethylsilyl-3-bromoallopurinol obtained by refluxing 109 in HMDS, was reacted with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (3) in the presence of trimethylsilyl trifluoromethanesulfonate in anhydrous CH_3CN at room temperature for 24 hr. A mixture of three nucleoside products resulted. Separation was readily achieved on a silica gel column by HPLC techniques and the crystalline major product was tentatively identified as 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-3-bromoallopurinol (110) on the basis of spectral data. The isolation and identification of the other isomers/anomers is in progress.

Like pyrazofurin, formycin B is also a naturally occurring C-nucleoside antibiotic and unlike N-nucleosides, formycin B will not suffer enzymatic cleavage

and degradation by phosphorolysis. Therefore, the half-life of the drug should be increased. Our interest in this group of nucleosides stems from our own earlier work on the original structural elucidation of formycin and formycin B.[69] Formycin B has shown significant activity against influenza A_1 virus grown on chorioallantoic membrane.[70] The synthesis of purine-6-carboxamide nucleoside analog in formycin series is of special interest.

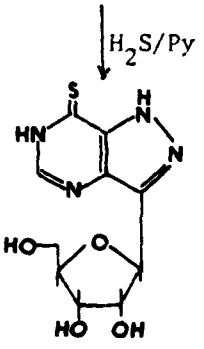
Deamination of formycin with aqueous nitrous acid at 55-60°C for 25 hr. provided an 85% yield of crystalline formycin B (BJ-86281). Acetylation of formycin B with a mixture of acetic anhydride and pyridine gave a good yield of 3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidin-7-one (111, BJ-86334). Chlorination of 111 with $POCl_3$ at 70-75°C gave a 70% yield of 7-chloro



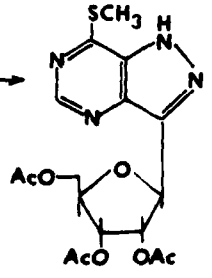
Formycin

Formycin B

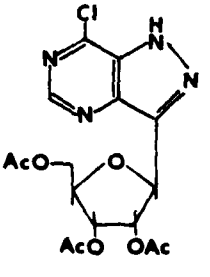
111



112



113

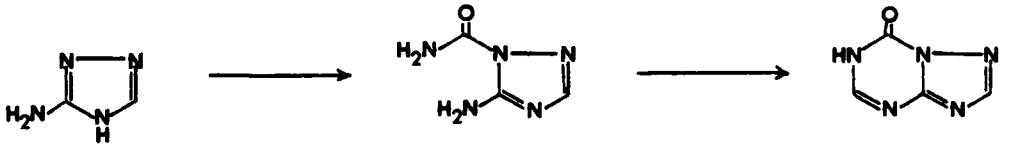


114

3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine (114). Direct nucleophilic displacement of the 7-chloro group by the treatment with methylmercaptan in the presence of stoichiometric amount of $KOBu^t$ gave the versatile inter-

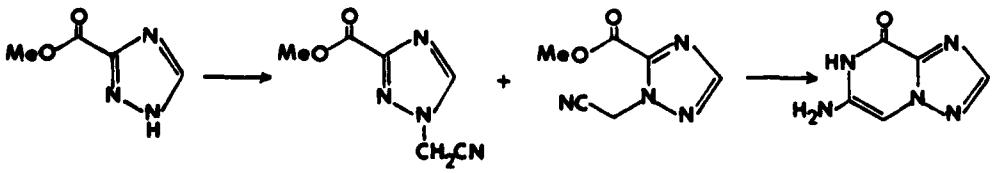
mediate 7-methylthio-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]-pyrimidine (113, BJ-91139), in rather low yield. 3-(β-D-Ribofuranosyl)pyrazolo-[4,3-d]pyrimidin-7-thione (thioformycin B, 112, BJ-63911) was prepared by sulfhydrolysis of formycin with liquid H_2S in anhydrous pyridine under elevated temperature and pressure, in low yield. The conversion of 113 to 3-(β-D-ribofuranosyl)-pyrazolo[4,3-d]pyrimidine-7-carboxamide, by following the sequence of reactions we recently established for the preparation of certain purine-6-carboxamide nucleosides is in progress.

Although many purine analogs must be converted to their ribosides in order to act as purine antagonists, numerous studies have shown that this is not a prerequisite for biological activity.[72] It even appears probable that, in certain cases, a potential purine antagonist may be rendered inactive by conversion in vivo to its riboside, and that retention of biological activity might result from blocking this conversion.[72] In view of these considerations, we prepared certain heterocyclic bases. Reaction of 3-amino-1,2,4-triazole (115) with potassium cyanate in acid solution gave 3-amino-2-carbamoyl-1,2,4-triazole



115 116 117

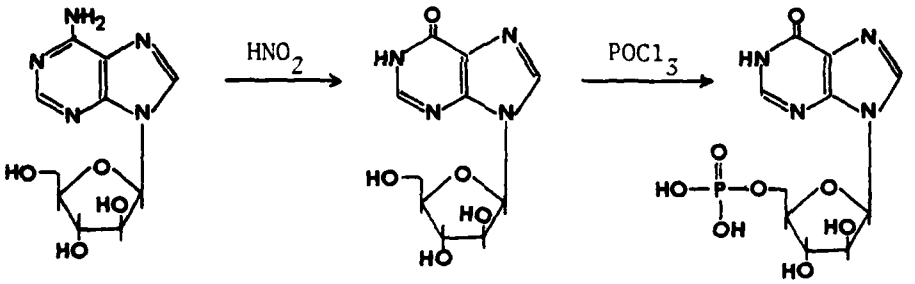
(116) in 78% yield.[73] Ring-annulation of 116 with triethyl orthoformate at reflux temperature gave s-triazolo[2,3-a]-s-triazin-4(5H)-one (5-azahypoxanthine, 117).[73] Similarly, alkylation of methyl 1,2,4-triazole-3-carboxylate (118)[28] with one mole



118 119 120 121

of iodoacetonitrile in DMF in the presence of K_2CO_3 at room temperature gave a mixture of two isomeric methyl 1-cyanomethyl-1,2,4-triazole-3-carboxylate (119) and the N_2-isomer (120).[74] These isomers were separated by column chromatography on silica gel, using $CHCl_3$:MeOH (20:1) as eluant. Treatment of 120 with MeOH/NH_3 should provide 6-amino-1,2,4-triazolo[1,5-a]pyrazin-4(5H)-one (3-deaza-4-azaguanine, 121).[74]

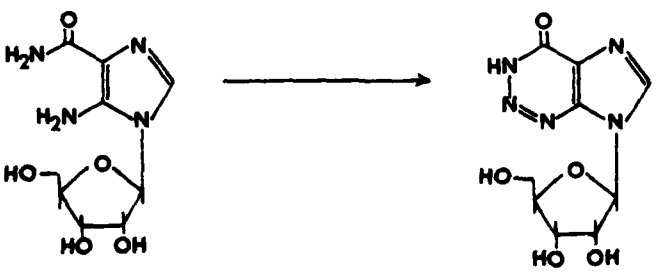
During the course of this synthetic study, certain purine type of nucleosides structurally related to BJ-42341 were also prepared. Compounds like arabinosylhypoxanthine, 2-azainosine and 8-azainosine may be viewed as fused 5-6 membered nucleosides in which the carboxamide function is an integral part of the molecule. Deamination of the antiviral agent ara-A with aqueous HNO_2 gave 9-(β-D-arabinofuranosyl)hypoxanthine (122, BJ-92467),[55] which on direct phosphorylation with $POCl_3$ furnished 9-(β-D-arabinofuranosyl)hypoxanthine



Ara-A 122 123

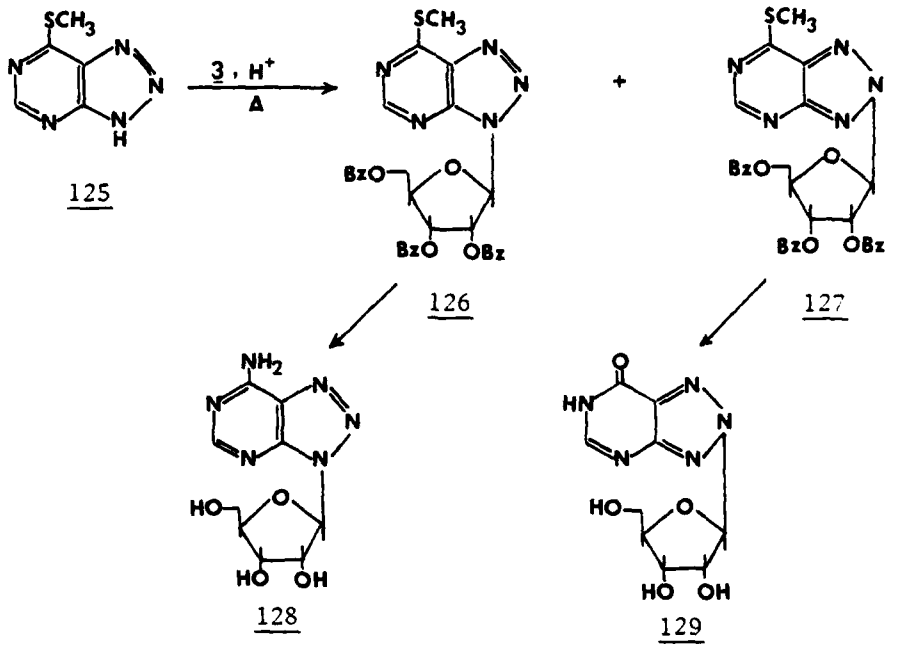
5'-phosphate (123, BJ-92476).[75]

Treatment of 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICAR) in 6N HCl at -25°C with 3N sodium nitrite solution gave 7-(β-D-ribofuranosyl)-imidazo[4,5-d]-v-triazin-4-one (2-azainosine, 124, BJ-92538) in 85% yield.[76]



AICAR 124

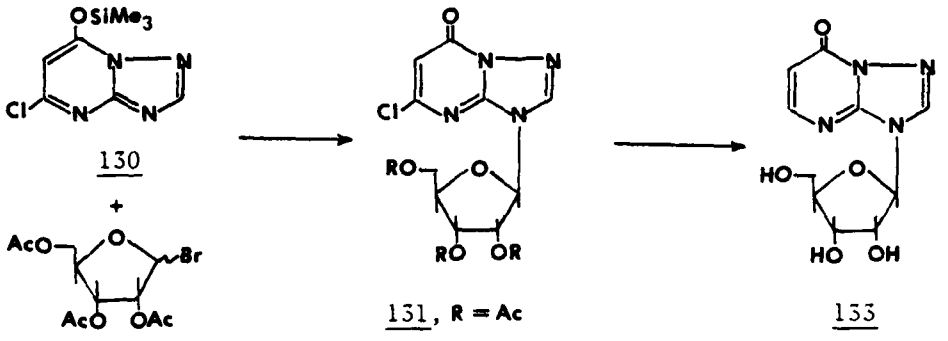
Acid catalyzed fusion of 7-methylthio-v-triazolo[4,5-d]pyrimidine (125)[77] with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose gave a mixture of two isomeric nucleosides, 7-methylthio-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-v-triazolo[4,5-d]pyrimidine (126) and the corresponding N_2-isomer (127),[78] which



125 126 127 128 129

were readily separated by silica gel column chromatography. Treatment of 126 with $MeOH/NH_3$ gave the adenosine analog 7-amino-3-(β-D-ribofuranosyl)-v-triazolo-[4,5-d]pyrimidine (128, BJ-76301).[78] Treatment of syrupy 127 with H_2O_2 in glacial acetic acid followed by NaOMe gave 2-(β-D-ribofuranosyl)-v-triazolo[4,5-d]pyrimidin-7-one (129, BJ-92501).[78]

We recently described[79] the first chemical synthesis of a purine nucleoside analog containing a bridgehead nitrogen atom. The aglycon moiety, 1,2,4-triazolo-[1,5-a]pyrimidine, can be pictured as purine in which N_1 and C_5 are interchanged. The synthesis of the inosine analog (133) was achieved as follows. Condensation of the trimethylsilyl derivative of 5-chloro-1,2,4-triazolo[1,5-a]pyrimidin-7-one (130) with 2,3,5-tri-O-acetyl-D-ribofuranosyl bromide in CH_3CN gave only one isolable



130

131, R = Ac

132, R = H

133

blocked nucleoside 131. Deacetylation of 131 with MeOH/NH_3 furnished 5-chloro-3-(β-D-ribofuranosyl)-1,2,4-triazolo[1,5-a]pyrimidin-7-one (132). Dehalogenation of 132 with Pd/C gave the inosine analog 3-(β-D-ribofuranosyl)-1,2,4-triazolo-[1,5-a]pyrimidin-7-one (133).[79] It is of particular interest to see the antiviral activity of 133.

Antiviral Evaluation:

Most of the compounds synthesized during the report period were tested at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, against RVFV, VEE, PICH, VF and SF viruses in vitro as well as in vivo. Of the compounds tested, in parallel with ribavirin, ribavirin 5'-phosphate (BJ-08456) and ribavirin 3',5'-cyclic phosphate (BJ-58536) were found to be equally active against PICH virus. However, 2',3'-di-O-acetylribavirin 5'-phosphate (BJ-22483) was active against SF virus in vitro. 5'-O-Butyryl- (BJ-45529), 5'-O-valeryl- (BJ-45548) and 5'-O-caproyl- (BJ-45539) were all inhibited PICH virus in vitro and the activity was comparable to that of ribavirin. Although 5-hydroxy-1,2,3-triazole-4-carboxamide (BJ-86325) and 8-azaadenosine (BJ-76301) exhibited significant antiviral activity against PICH virus in vitro, formycin B (BJ-86281) was found to be more efficacious than ribavirin against PICH virus. Both 3-(β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidin-7-thione (thioformycin B, BJ-63911)

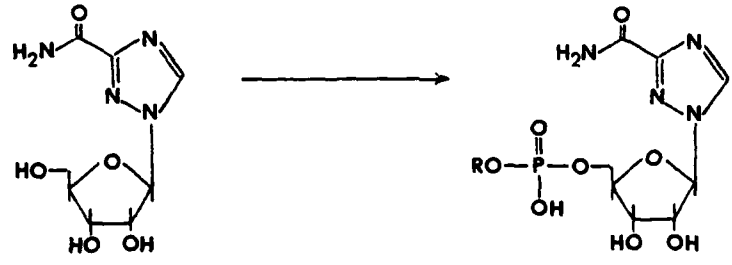
and 7-methylthio-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine (BJ-91139) showed significant activity against YF virus. In addition to YF virus, thioformycin B was active against SF virus in vitro.

5-Chloro-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (BJ-42350), 9-(β-D-ribofuranosyl)purine-6-carbonitrile (BJ-63939) and 9-(β-D-ribofuranosyl)purine-6-carboxamide (BJ-42341) were all significantly active against RVFV. 9-(β-D-Ribofuranosyl)purine-6-carboxamide 5'-monophosphate (BJ-81544) and 9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine-6-thiocarboxamide (BJ-76221) showed moderate antiviral activity against YF and SF. While 9-(β-D-ribofuranosyl)purine-6-thiocarboxamide (BJ-81526) and 4-amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-d]-pyrimidine (BJ-76187) were both highly active against YF and SF, BJ-76187 was found to be more efficacious than ribavirin against YF in vitro. 3-Nitro (or amino)-1-(β-D-ribofuranosyl)-1,2,4-triazole (BJ-91111 and BJ-91068), the C-nucleoside analog of ribavirin (BJ-45502), allopurinol riboside (BJ-86290), thioallopurinol riboside (BJ-63868), 6-methylthio-7-deazapurine riboside (BJ-63886) and 4-amino-8-(β-D-arabinofuranosylamino)pyrimido[5,4-d]pyrimidine (BJ-91077) were all essentially inactive against the viruses employed.

It is of considerable interest that 7-deazainosine (BJ-84125), 2',3',5'-tri-O-acetyl-7-deazainosine (BJ-84134), 6-chloro-7-deazapurine riboside (BJ-84145), 4-methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5-carbonitrile (BJ-76294) and 4-chloro-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (BJ-76276) showed very significant antiviral activity against all the viruses used. 4-Chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (BJ-84143) was found to be superior to ribavirin against RVFV, PICH, YF and SF in vitro and the activity was comparable to that of pyrazofurin or tunicamycin. However, like pyrazofurin and tunicamycin, BJ-84143 was found to be toxic in vivo.

Work in Progress:

The inhibition of various viral nucleic acid enzymes is assumed to occur at the nucleotide level. However, direct administration of pre-formed nucleotides is generally not practical due to the ionic phosphate moiety which renders poor cellular penetration.[80] A practical approach may be to utilize 5'-O-alkylphosphate derivatives of nucleosides in the hope that these lipophilic prodrug forms would enter cells by passive diffusion and thereupon be cleaved enzymatically to the free nucleotides.[81] Presently the preparation of 5'-O-alkylphosphate derivatives of ribavirin (134) is in progress. Direct phosphorylation of ribavirin



Ribavirin

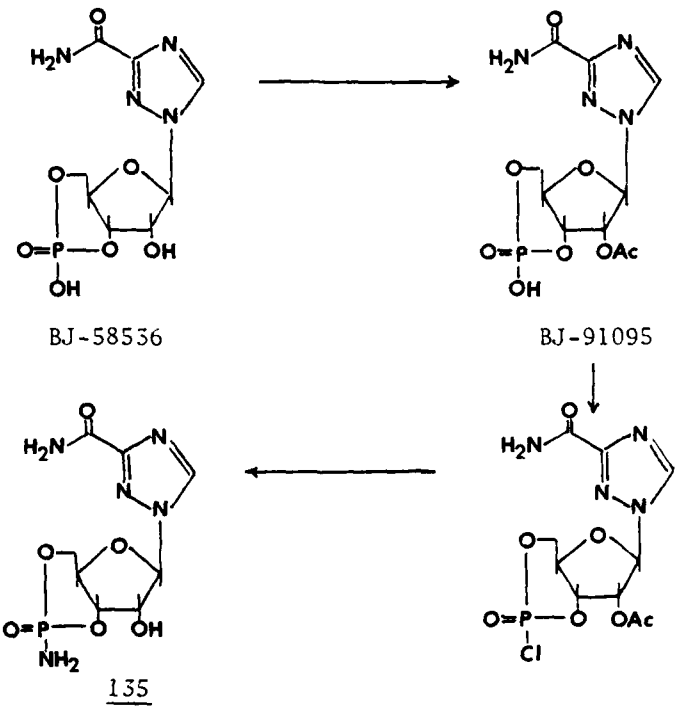
134

R = Me, Et

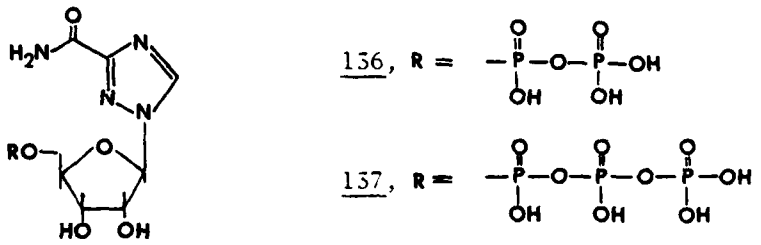
with alkyl phosphorodichloridate[75] or with $POCl_3$ followed by quenching the reaction mixture with appropriate alcohol[82] should give 134.

One of the most exciting results of in vivo testing of ribavirin derivatives prepared under the subject contract is the pronounced antiviral activity of ribavirin 3',5'-cyclic phosphate (BJ-58536) against VEE. Perhaps one of the important structural features of nucleoside 3',5'-cyclic phosphates is the ionic hydroxyl attached to the 3',5'-cyclic phosphate ring. In order to further lower the ionic nature of the cyclic phosphate ring, substitution of the phosphate hydroxyl by NH_2 has been undertaken. Such a replacement of an exocyclic OH by NH_2 to obtain ribavirin 3',5'-cyclic phosphoramidate (135) is being accomplished by the procedure previously reported by Robins and co-workers.[83] Acetylation of BJ-58536 with acetic anhydride in pyridine gave the 2'-O-acetyl derivative (BJ-91095), which

on further heating with thionyl chloride followed by treatment with NH_3 should provide 135.



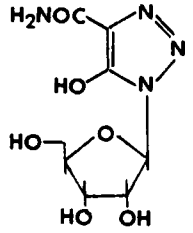
The preparation of ribavirin 5'-diphosphate (136) and the 5'-triphosphate (137), required for the proposed biochemical studies, is also in progress. These di- and tri-phosphates of ribavirin will be chemically synthesized by following the general procedure of Moffatt and Khorana.[84] Ribavirin 5'-phosphoromorpholidate



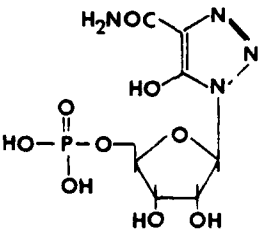
will be prepared from ribavirin 5'-phosphate (BJ-08456), which will be subsequently reacted with orthophosphoric acid to obtain 136. Similar reaction with pyrophosphate should give the triphosphate 137.

The synthesis of the N-nucleoside analog of pyrazofurin, 5-hydroxy-1-(β-D-ribofuranosyl)-1,2,3-triazole-4-carboxamide (35) and the corresponding 5'-phosphate

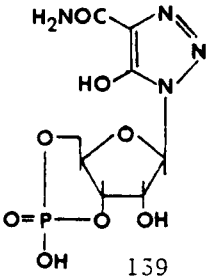
(138) as well as the 3',5'-cyclic phosphate (139) is in progress. The synthesis of 35 is being achieved by the glycosylation of the trimethylsilyl derivative of 4(5)-hydroxy-1,2,3-triazole-5(4)-carboxamide (BJ-86325) with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in presence of trimethylsilyl trifluoromethanesulfonate as a catalyst. Compound 35 will then be converted to 139 by the conventional procedure via the 5'-phosphate (138).



35

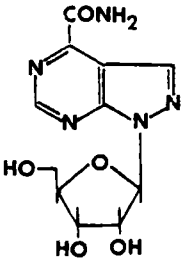


138

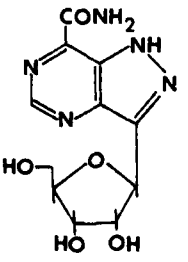


139

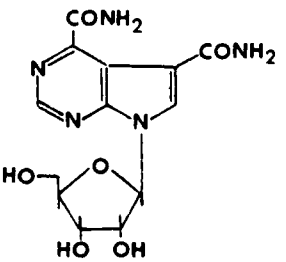
The isolation and identification of all the three nucleoside products formed during the glycosylation of 3-bromoallopurinol (109) is also in progress. In addition, the synthesis of other purine nucleoside analogs containing the carboxamide, such as 140, 141 and 143 is in progress.



140



141



142

The project on the chemical synthesis of derivatives of pyrazofurin and fused 5-5 membered nucleosides in which the carboxamide function is an integral part of a ring system will be initiated.

Biochemical Studies

1. cAMP and Ribavirin 3',5'-Cyclic Phosphate as Stimulators of 2',5'-Oligo (adenylate) Synthetase and eIF-2 Protein Kinase

Research efforts have been directed at investigating the mechanism of action of ribavirin 3',5'-cyclic phosphate (cRMP) as outlined in the proposal. cRMP had been found to have exceptionally good antiviral activity in VEE infections in mice at Fort Detrick in preliminary studies, and it was postulated that cRMP might act as part of a hormonal response similar to that postulated for the broad spectrum antiviral agent, interferon. Interferon is known to raise cAMP levels,[85-89] and also there is one published report for increases in the level of cGMP in cells treated with interferon.[90] Interferon has more than one possible mechanism of action.[91,92] For example, interferon is known to raise the levels of 2',5'-oligo(adenylate) synthetase and a protein kinase that phosphorylates eIF-2.[93] 2',5'-Oligo(adenylate) synthetase uses 5'-ATP as a substrate for the synthesis of 2'→5'-pppApApA (predominantly as the trimer),[94] which is then believed to activate a latent endoribonuclease that selectively degrades viral mRNA[95-97] and perhaps also rRNA.[98] The phosphorylation of eIF-2 is thought to block the formation of the initiation complex, thereby shutting off protein synthesis.[93,99-103] eIF-2 forms a ternary complex with 40S ribosomal subunits and met-tRNAs that is essential for starting up protein biosynthesis in the cell. The proposal suggested that either cAMP or cGMP might be secondary messengers for interferon that lead to the stimulation of the 2',5'-oligo(adenylate) synthetase and eIF-2 protein kinase and that cRMP might substitute in the cell for the cAMP or cGMP. Ribavirin seems to resemble adenosine since it can be used as a substrate by adenosine kinase,[104,105] and it also seems to resemble guanosine in single crystal x-ray[106] and by its effect diminishing intracellular pools of guanosine nucleotides.[107]

We have succeeded in setting up the assay systems for 2',5'-oligo(adenylate) synthetase and eIF-2 protein kinase to test for the possible stimulatory effects

of cRMP. Mouse L cell enzymes isolated from cells treated with mouse fibroblast interferon (200 units/ml for 24 h) were studied in comparison to nontreated controls for enzyme stimulation. 2',5'-Oligo(adenylate) synthetase was partially purified from mouse L cells in the presence and absence of interferon and assayed bound to polyI:polyC-agarose according to a modification of the procedure of Kimchi et al.[93] The interferon treated cells showed a 20-fold increase in the levels of the enzyme over the untreated controls in our Laboratory. The [32p]-labeled oligo(adenylate) product was analyzed by paper electrophoresis,[108] thin layer chromatography on PEI sheets,[109] and by HPLC using a newly developed gradient system that gave better separation than any reported in the literature. All three methods gave identical results in terms of the quantities of dimer, trimer and tetramer produced. The eIF-2 protein kinase activity was measured in cell extracts for interferon treated versus control cells, and the eIF-2 phosphorylation was found to be stimulated 6-fold in the case of interferon in our hands, which is in agreement with published values.[93] eIF-2 was prepared according to the procedure of Odom et al.[110] as learned in Dr. Boyd Hardesty's laboratory this spring.

In order to examine cRMP's possible role in stimulating 2',5'-oligo(adenylate) synthetase and eIF-2 protein kinase, studies were first started with cAMP in order to determine if it could be a secondary messenger for the hormone-like activity of interferon. Cells were treated with cAMP at 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, or 10^{-9} M for 24 h, cell extracts were prepared and the relative enzyme activities determined. No stimulation above the control levels was found, so further studies on cAMP were carried out using cAMP at these same concentrations and activities were scored at 0, 2, 4, 6, 8, 10, 24 and 30 h again with no detectable increase in the activity of either enzyme. It seemed possible that cAMP was being degraded too rapidly to show the effect, so various cAMP phosphodiesterase inhibitors were added together with cAMP at the concen-

trations indicated and for various times (0, 2, 4, 6, 8, 10, and 24 h). Theophylline (1 mM),[111-113] 1-methyl-3-isobutyl xanthine (MIX) (1 mM),[114] or 4-(butoxy-4-methoxybenzyl)-2-imidazolidine (RO 20-1724) (a gift of Dr. Herbert Sheppard of Hoffman-LaRoche, Inc.)[115] were studied since all have been shown to increase cellular cAMP levels by inhibiting cAMP phosphodiesterase activity. These were examined in the presence and absence of cAMP (10^{-3} to 10^{-8} M, as above) for various times (0 to 30 h, as above) but without noticeable effect on either of the two enzymes.

cGMP was also examined as a possible secondary messenger responsible for the stimulation of 2',5'-oligo(adenylate) synthetase or eIF-2 protein kinase. 1 mM cGMP was incubated with cells in a kinetic study over the time range, 0 to 30 h, but no stimulation resulted for the two enzymes. In view of the fact that Tovey et al.[90] observed that both cGMP and cAMP levels were increased with interferon treatment, we also performed an experiment involving the addition of 1 mM cGMP along with 1 mM theophylline which should then lead to increases in both cGMP and cAMP in the cells. No effect was found on either of the two enzyme activities.

There are other agents known to raise cAMP levels, e.g., PGE_1,[116,117] cholera toxin,[118] and epinephrine,[119] two of which show antiviral activity in mouse L cells, so cells were also treated with these. PGE_1 at 50 µg/ml raises cAMP levels 10-fold starting at 5 min up to 1 h and then a 2-fold stimulation is sustained for up to 24 h.[116] Cells treated with PGE_1 (50 µg/ml) over this time period did not show increased enzyme activities in our Laboratory. Cholera toxin (10^{-7} to 10^{-9} M) in the presence of 30 units/ml interferon raises cAMP levels and also decreased EMC virus titers by 50%.[118] Cells treated in a like manner did not show any effect on either 2',5'-oligo(adenylate) synthetase or eIF-2 protein kinase. Cells treated with epinephrine (10^{-3} M) and MIX (10^{-3}M) for 4 h and then with low concentrations of interferon (0.5 to 2 units/ml) for

16 h showed a 67% decrease in VSV titers according to Goldberg et al.[119] They also showed a 1.3-fold increase in cAMP levels for epinephrine (10^{-3} M) and MIX (10^{-3} M) after 4 h. In our Laboratory, cells treated either with epinephrine (10^{-3} M) and MIX (10^{-3}M) for 4 h or by epinephrine (10^{-3} M) and MIX (10^{-3} M) for 4 h followed by interferon (0.5 to 60 units/ml) for 16 h did not show any stimulation of either 2',5'-oligo(adenylate) synthetase or eIF-2 protein kinase.

It was then considered that interferon might have another effect (such as its known perturbation of the cell membrane) that might be important for its antiviral action, so we decided to add interferon at suboptimal concentrations along with the cAMP or cRMP. Interferon (5 to 10 units/ml) was added together with cAMP (10^{-3} to 10^{-10}, as previously) for 24 h, but no detectable increase was observed for the enzyme activities. We then examined cRMP, which it was hoped might be more stable than cAMP, as the possible stimulator for these two enzymes. cRMP was added in the range of concentrations from 10^{-3} to 10^{-10} along with 5 units/ml interferon for 24 h and the enzymes were assayed. No stimulation was observed over the basal levels.

The fact that no evidence was found for involvement of cAMP, cGMP or cRMP in the stimulation of the enzymes, 2',5'-oligo(adenylate) synthetase or eIF-2 protein kinase still does not preclude their involvement in these antiviral pathways. It could be that their actions in the cell are highly compartmentalized and that the cAMP, cGMP or cRMP do not arrive at the specific target required for their action when introduced into whole cells. The facts that PGE_1, cholera toxin and epinephrine raise cAMP levels but do not show any effect on these two enzymes probably indicates how highly directionalized these reactions may be. It is also possible that the enzyme activation requires some other factor from the virus (e.g., double-stranded RNA--a known activator for both 2',5'-oligo-(adenylate) synthetase and eIF-2 protein kinase)[91,92] that sets the whole process, i.e., the possible cascade effect, in motion. We plan, therefore, to

continue with a similar set of experiments to these using virus infected cells. We also plan to study the involvement of cRMP in the viral mRNA "capping" mechanism since some recent reports have indicated that this might be a mechanism for ribavirin's antiviral activity.[120-122]

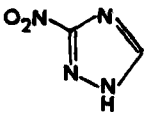
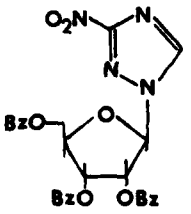
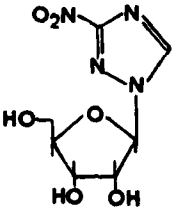
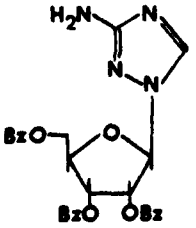
Preparation of Single-stranded Polymers of Ribavirin or Ribavirin Analogs as Possible New Antiviral Agents

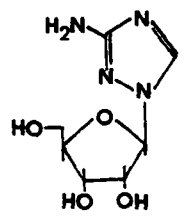
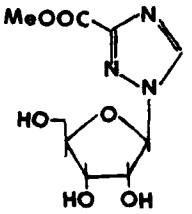
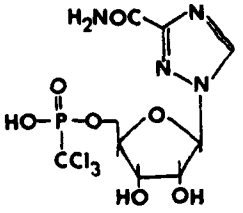
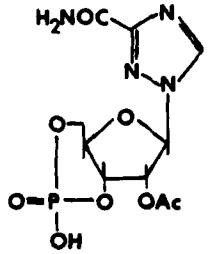
Single-stranded polymers of ribavirin or its analogs may be very useful antiviral agents since they may be slowly released in the cell and, in addition, they may have antiviral activity themselves. Some recent papers have reported antiviral activity for single-stranded RNA-like 3'→5'-linked polymers.[123,124] 3'→5'-Linked polymers of ribavirin will be prepared using polynucleotide phosphorylase (PNPase). We now have PNPase on hand from E. coli which is known to polymerize GDP[125] and from M. luteus which is known to polymerize ADP.[126] Reaction conditions suitable for ribavirin diphosphate polymerization are being studied. These conditions will also be used to polymerize other ribavirin analogs which will then be studied as antiviral agents under the direction of the project officer, Dr. P. Canonico. Dr. Arthur Brown, a former student of Dr. Robins, at the University of Utah has recently succeeded in polymerizing ribavirin 5'-diphosphate and has furnished us with some of this polymer for preliminary antiviral studies. Polymerization of ribavirin and ribavirin analogs should be possible in the near future in our Laboratory then due to the finding that ribavirin 5'-diphosphate can in fact be polymerized by PNPase.

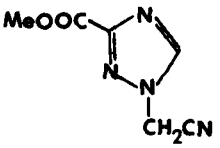
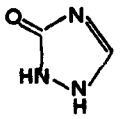
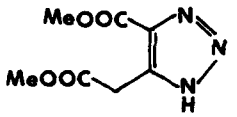
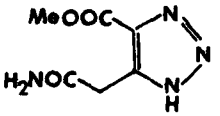
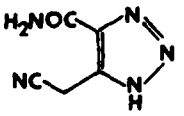
We also plan to synthesize 2'→5'-linked oligomers of ribavirin and its analogs, since these also have great potential as antiviral agents. 2'→5'-Linked oligoadenylate (2'→5'-oligo A) has been shown to have antiviral activity.[127] 2'→5'-Oligo A has also been shown recently to prevent viral mRNA methylation similarly to ribavirin itself, and this may also be responsible for its antiviral activity.[121] Presently, we have partially purified the enzyme

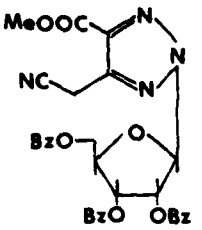
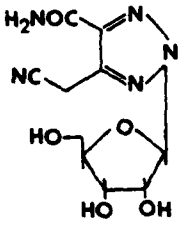
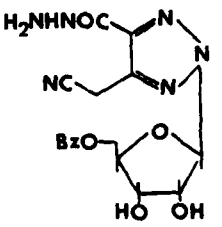
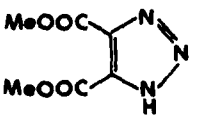
2'→5'-oligo A synthetase and prepared 2'→5'-oligo A according to published procedures.[128] These conditions will also be used to polymerize ribavirin 5'-triphosphate when it becomes available.

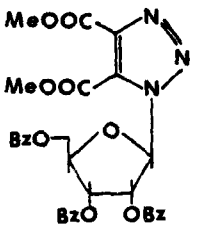
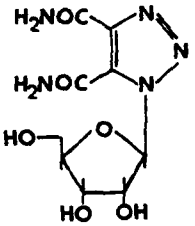
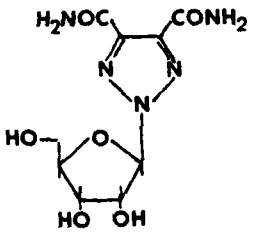
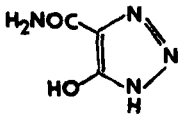
The following eighty-one compounds have been prepared and submitted to Medical Research and Development Command, Walter Reed Medical Center, U.S. Army, for antiviral evaluation, each in pure form. The chemical structure of each of these compounds is shown below:

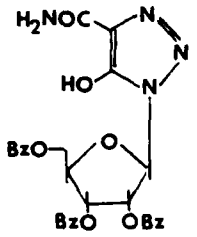
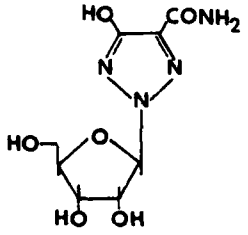
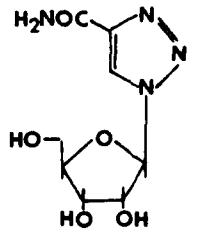
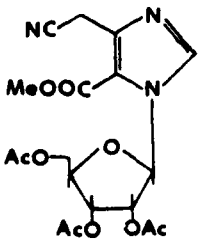
No.	Compound	Notebook No.	WRAIR No.	Ref.
1.	3-Nitro-1,2,4-triazole 	RA-189	BJ-91059	Ref. 25
2.	3-Nitro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazole 	RV-177	BJ-91111	Ref. 26
3.	3-Nitro-1-(β-D-ribofuranosyl)-1,2,4-triazole 	RV-176	BJ-91102	Ref. 26
4.	3-Amino-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,4-triazole 	RV-178	BJ-91120	p. 8

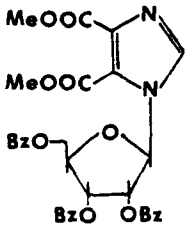
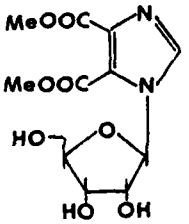
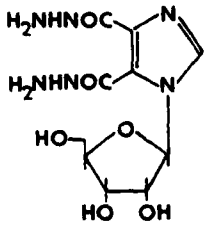
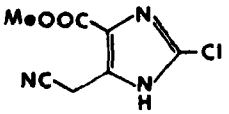
No.	Compound	Notebook No.	WRAIR No.	Ref.
5.	3-Amino-1-(β-D-ribofuranosyl)-1,2,4-triazole 	RA-190	BJ-91068	Ref. 26
6.	Methyl 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate 	RA-135	BJ-81517	Ref. 9
7.	1-(β-D-Ribofuranosyl)-1,2,4-triazole-3-carboxamide 5'-trichloromethylphosphonate 	RY-84	BJ-92529	p. 10
8.	1-(2-O-Acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide 3',5'-cyclic phosphate 	RV-171	BJ-91095	p. 10

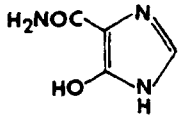
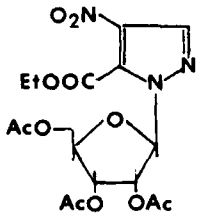
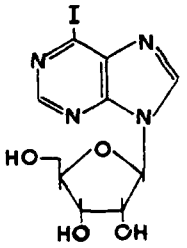
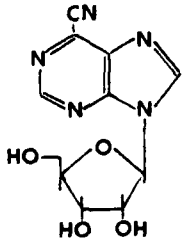
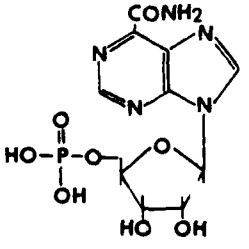
No.	Compound	Notebook No.	WRAIR No.	Ref.
9.	Methyl 1-cyanomethyl-1,2,4-triazole-3-carboxylate 	RA-211	BJ-	Ref. 74
10.	1,2,4-Triazolin-3-one 	RV-203	BJ-	Ref. 43
11.	Methyl 4(5)-methoxycarbonylmethyl-1,2,3-triazole-5(4)-carboxylate 	RA-175	BJ-84170	Ref. 34
12.	Methyl 4(5)-carbamoylmethyl-1,2,3-triazole-5(4)-carboxylate 	RA-212	BJ-	Ref. 34
13.	4(5)-Cyanomethyl-1,2,3-triazole-5(4)-carboxamide 	RA-176	BJ-84189	Ref. 34

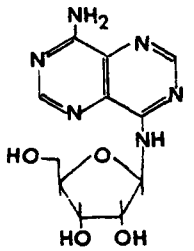
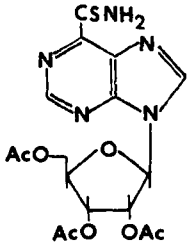
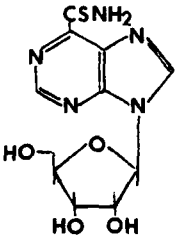
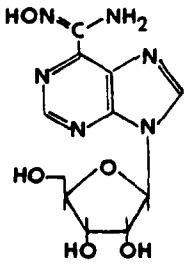
No.	Compound	Notebook No.	WRAIR No.	Ref.
14.	Methyl 5-cyanomethyl-2-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,3-triazole-4-carboxylate 	RA-33	BJ-84198	Ref. 34
15.	5-Cyanomethyl-2-(β-D-ribofuranosyl)-1,2,3-triazole-4-carboxamide 	RA-36	BJ-84205	Ref. 34
16.	5-Cyanomethyl-2-(5-O-benzoyl-β-D-ribofuranosyl)-1,2,3-triazole-4-carboxhydrazide 	RA-42	BJ-	p. 12
17.	4,5-Dicarbomethoxy-1,2,3-triazole 	RA-194	BJ-92449	Ref. 36

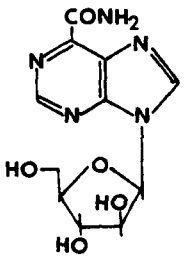
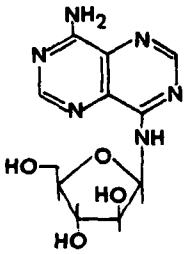
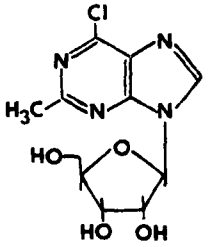
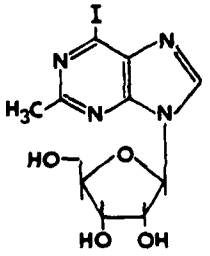
No.	Compound	Notebook No.	WRAIR No.	Ref.
18.	Dimethyl 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,3-triazole-4,5-dicarboxylate 	RA-199	BJ-92485	Ref. 38
19.	1-(β-D-Ribofuranosyl)-1,2,3-triazole-4,5-dicarboxamide 	RA-200	BJ-92494	Ref. 38
20.	2-(β-D-Ribofuranosyl)-1,2,3-triazole-4,5-dicarboxamide 	RA-196	BJ-92458	p. 17
21.	5-Hydroxy-1,2,3-triazole-4-carboxamide 	RY-29	BJ-86325	Ref. 40

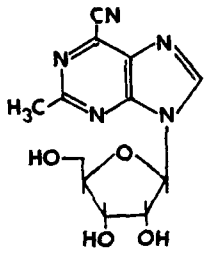
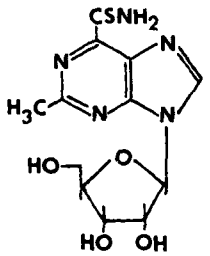
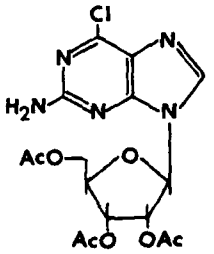
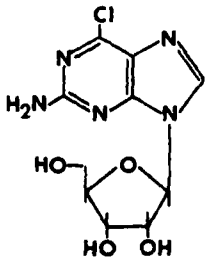
No.	Compound	Notebook No.	WRAIR No.	Ref.
22.	5-Hydroxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2,3-triazole-4-carboxamide 	RV-190	BJ-92556	p. 14
23.	5-Hydroxy-2-(β-D-ribofuranosyl)-1,2,3-triazole-4-carboxamide 	RV-189	BJ-92547	p. 14
24.	1-(β-D-Ribofuranosyl)-1,2,3-triazole-4-carboxamide 	RA-159	BJ-81535	Ref. 42
25.	Methyl 4-cyanomethyl-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylate 	RA-187	BJ-91040	Ref. 44

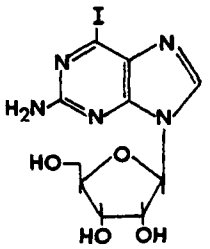
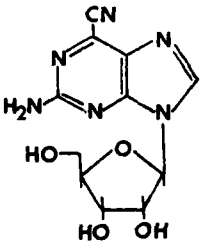
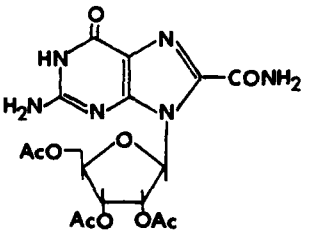
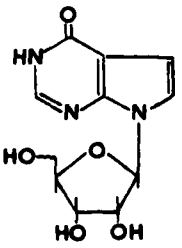
No.	Compound	Notebook No.	WRAIR No.	Ref.
26.	Dimethyl 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazole-4,5-dicarboxylate 	RA-174	BJ-84161	Ref. 45
27.	Dimethyl 1-(β-D-ribofuranosyl)imidazole 4,5-dicarboxylate 	RP-91	BJ-81553	Ref. 45
28.	1-(β-D-Ribofuranosyl)imidazole-4,5-dicarboxhydrazide 	RA-210	BJ-	Ref. 45
29.	Methyl 5(4)-Cyanomethyl-2-chloro-imidazole-4(5)-carboxylate 	RA-209	BJ-	Ref. 33

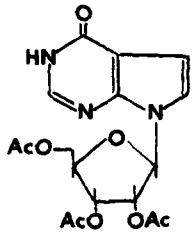
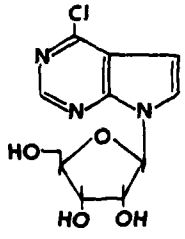
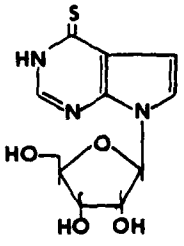
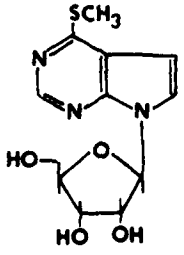
No.	Compound	Notebook No.	WRAIR No.	Ref.
30.	5-Hydroxyimidazole-4-carboxamide 	RA-202	BJ-92510	Ref. 47
31.	5-Ethoxycarbonyl-4-nitro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole 	RP-77	BJ-76267	Ref. 48
32.	9-(β-D-Ribofuranosyl)-6-iodopurine 	RV-57	BJ-63920	Ref. 52
33.	9-(β-D-Ribofuranosyl)-6-cyanopurine 	RI-91	BJ-63939	p. 20
34.	9-(β-D-Ribofuranosyl)purine-6-carboxamide 5'-monophosphate 	RV-116	BJ-81544	p. 20

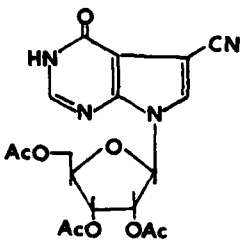
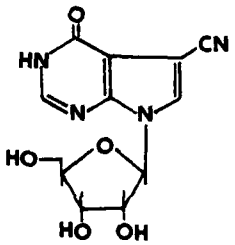
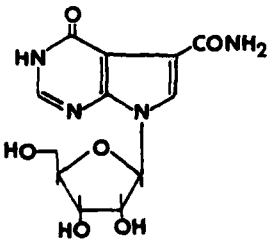
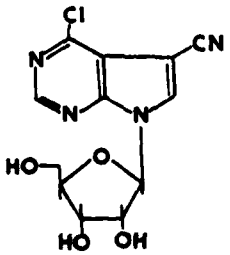
No.	Compound	Notebook No.	WRAIR No.	Ref.
35.	4-Amino-8-(β-D-ribofuranosylamino)-pyrimido[5,4-d]pyrimidine 	RA-149	BJ-76187	Ref. 53
36.	9-(2,3,5-Tri-O-acetyl-β-D-ribofurano-syl)purine-6-thiocarboxamide 	RI-25	BJ-76221	p. 20
37.	9-(β-D-Ribofuranosyl)purine-6-thio-carboxamide 	RA-155	BJ-81526	p. 21
38.	9-(β-D-Ribofuranosyl)purine-6-carboxa-midoxime 	RA-137	BJ-63902	p. 21

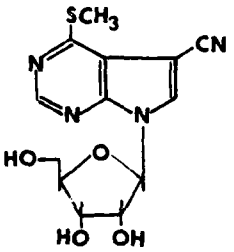
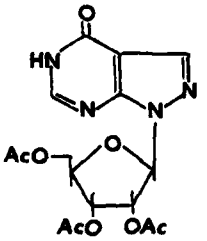
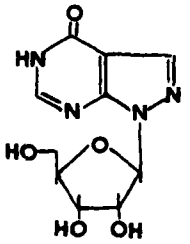
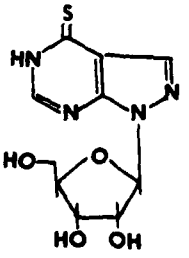
No.	Compound	Notebook No.	WRAIR No.	Ref.
39.	9-(β-D-Arabinofuranosyl)purine-6-carboxamide 	RA-192	BJ-91086	Ref. 56
40.	4-Amino-8-(β-D-arabinofuranosylamino)-pyrimido[5,4-d]pyrimidine 	RA-191	BJ-91077	Ref. 56
41.	2-Methyl-9-(β-D-ribofuranosyl)-6-chloropurine 	RA-152	BJ-76203	Ref. 57
42.	2-Methyl-9-(β-D-ribofuranosyl)-6-iodopurine 	RI-61	BJ-76230	p. 23

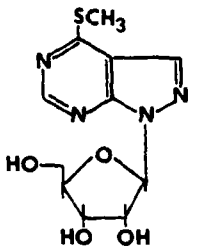
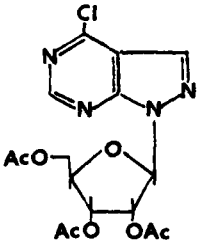
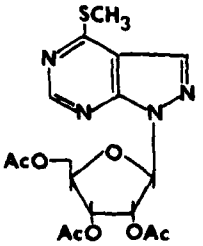
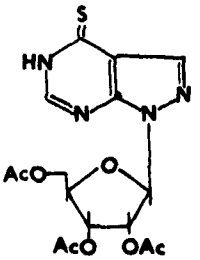
No.	Compound	Notebook No.	WRAIR No.	Ref.
43.	2-Methyl-9-(β-D-ribofuranosyl)-6-cyanopurine 	RI-63	BJ-76249	p. 23
44.	2-Methyl-9-(β-D-ribofuranosyl)purine-6-thiocarboxamide 	RI-66	BJ-76258	p. 23
45.	2-Amino-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-6-chloropurine 	RA-151	BJ-76196	Ref. 52
46.	2-Amino-9-(β-D-ribofuranosyl)-6-chloropurine 	RI-36	BJ-63948	Ref. 52

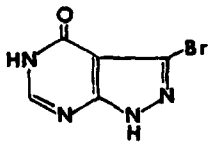
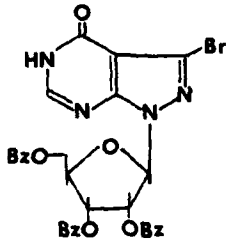
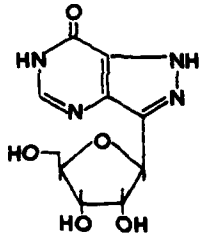
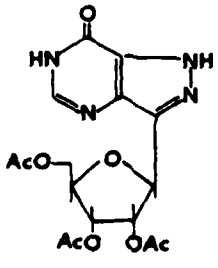
No.	Compound	Notebook No.	WRAIR No.	Ref.
47.	2-Amino-9-(β-D-ribofuranosyl)-6-iodopurine 	RI-33	BJ-63957	p. 23
48.	2-Amino-9-(β-D-ribofuranosyl)-6-cyano-purine 	RV-66	BJ-63966	p. 23
49.	2',3',5'-Tri-O-acetyl-8-carbamoyl-guanosine 	RI-20	BJ-76212	p. 23
50.	7-(β-D-Ribofuranosyl)pyrrolo[2,3-d]-pyrimidin-4-one 	RV-133	BJ-84125	Ref. 59

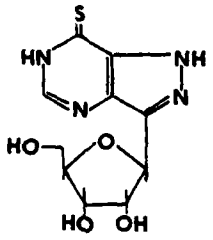
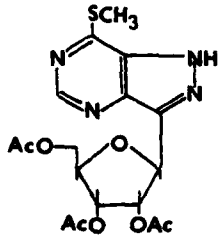
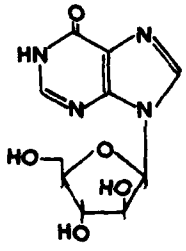
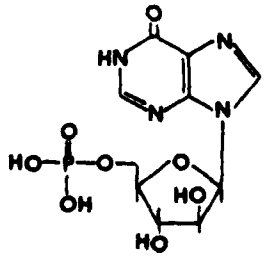
No.	Compound	Notebook No.	WRAIR No.	Ref.
51.	7-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidin-4-one 	RA-66	BJ-84134	Ref. 60
52.	4-Chloro-7-(β-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidine 	RA-71	BJ-84143	Ref. 60
53.	7-(β-D-Ribofuranosyl)pyrrolo[2,3-d]-pyrimidin-4-thione 	RA-72	BJ-84152	Ref. 60
54.	4-Methylthio-7-(β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine 	RA-73	BJ-63886	Ref. 60

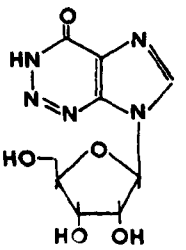
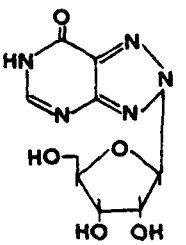
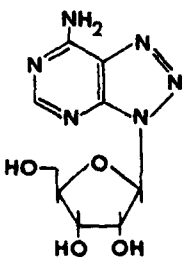
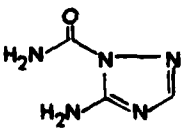
No.	Compound	Notebook No.	WRAIR No.	Ref.
55.	5-Cyano-7-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidin-4-one 	RA-181	BJ-86307	Ref. 61
56.	5-Cyano-7-(β-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidin-4-one 	RP-75	BJ-63895	Ref. 61
57.	5-Carbamoyl-7-(β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidin-4-one 	RV-198	BJ-	Ref. 61
58.	4-Chloro-5-cyano-7-(β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine 	RP-80	BJ-76276	Ref. 62

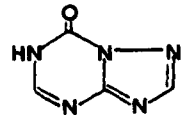
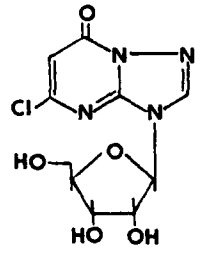
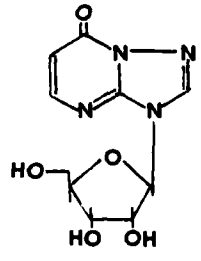
No.	Compound	Notebook No.	WRAIR No.	Ref.
59.	4-Methylthio-7-(β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine-5-carbonitrile 	RP-83	BJ-76294	Ref. 62
60.	1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidin-4-one 	RY-2	BJ-84090	Ref. 65
61.	1-(β-D-Ribofuranosyl)pyrazolo[3,4-d]-pyrimidin-4-one 	RA-180	BJ-86290	Ref. 65
62.	1-(β-D-Ribofuranosyl)pyrazolo[3,4-d]-pyrimidin-4-thione 	RA-148	BJ-63868	Ref. 66

No.	Compound	Notebook No.	WRAIR No.	Ref.
63.	4-Methylthio-1-(β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine 	RA-150	BJ-63877	Ref. 66
64.	4-Chloro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine 	RY-22	BJ-84107	p. 28
65.	4-Methylthio-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine 	RV-126	BJ-84116	p. 28
66.	1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidin-4-thione 	RY-6	BJ-86316	Ref. 66

No.	Compound	Notebook No.	WRAIR No.	Ref.
67.	3-Bromopyrazolo[3,4-d]pyrimidin-4-one 	RY-91	BJ-	Ref. 68
68.	3-Bromo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidin-4-one 	RY-99	BJ-	p. 29
69.	3-(β-D-Ribofuranosyl)pyrazolo[4,3-d]-pyrimidin-7-one 	RA-138	BJ-86281	p. 30
70.	3-(2,3,5-Tri-O-acetyl-β-D-ribofurano-syl)pyrazolo[4,3-d]pyrimidin-7-one 	RY-43	BJ-86334	Ref. 71

No.	Compound	Notebook No.	WRAIR No.	Ref.
71.	3-(β-D-Ribofuranosyl)pyrazolo[4,3-d]-pyrimidin-7-thione 	RV-24	BJ-63911	p. 31
72.	7-Methylthio-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-d]-pyrimidine 	RY-59	BJ-91139	p. 31
73.	9-(β-D-Arabinofuranosyl)hypoxanthine 	RA-197	BJ-92467	Ref. 55
74.	9-(β-D-Arabinofuranosyl)hypoxanthine 5'-monophosphate 	RA-198	BJ-92476	Ref. 75

No.	Compound	Notebook No.	WRAIR No.	Ref.
75.	7-(β-D-Ribofuranosyl)imidazo[4,5-d]-v-triazin-4-one 	RY-92B	BJ-92538	Ref. 76
76.	2-(β-D-Ribofuranosyl)-v-triazolo-[4,5-d]pyrimidin-7-one 	RA-201	BJ-92501	Ref. 78
77.	7-Amino-3-(β-D-ribofuranosyl)-v-triazolo[4,5-d]pyrimidine 	RP-86	BJ-76301	Ref. 78
78.	3-Amino-1,2,4-triazole-2-carboxamide 	RV-201	BJ-	Ref. 73

No.	Compound	Notebook No.	WRAIR No.	Ref.
79.	s-Triazolo[2,3-a]-s-triazin-4(5H)-one 	RV-202	BJ-	Ref. 73
80.	5-Chloro-3-(β-D-ribofuranosyl)-s-triazolo[1,5-a]pyrimidin-7-one 	RA-213	BJ-	Ref. 79
81.	3-(β-D-Ribofuranosyl)-s-triazolo-[1,5-a]pyrimidin-7-one 	RV-199	BJ-	Ref. 79

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